THE EFFECTS OF INDIVIDUAL AND COMBINATIONS OF AIRBORNE POLLUTANTS ON FEED INTAKE, IMMUNE FUNCTION AND PHYSIOLOGY OF THE PIG

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Chapter 3: The effects of ammonia and alpha haemolytic cocci (AHC) on feed intake, immune function and physiology in pigs

Summary

Poor air quality and surface hygiene are associated with increases in the prevalence and severity of enteric and respiratory diseases, as well as reduced growth rates in pigs. The pollutants which contribute to poor air quality include gases, dust, airborne particles, microorganisms and their toxins. In this study I investigated; 1) the effects of ammonia and alpha haemolytic cocci (AHC) including viridans-group streptococci (VGS) on feed intake, immune function and respiratory tract physiology in pigs, 2) the effects of stocking density on air quality parameters and growth rate in pigs and 3) the effects of shed design and management on air quality parameters. While exposure to AHC appeared to have a greater effect than ammonia on growth rate and feed efficiency, as well as aspects of immune function, the most significant effects were observed in pigs exposed to high levels of ammonia followed by AHC.

There was a strong positive relationship between the stocking density (StD) (m^3 airspace/pig) and the mean growth rate in pigs from 10 to 22 weeks of age, in an all-in/all-out (AIAO) system. There was also a strong negative relationship between stocking density and the number of viable bacteria in the airspace. As the volume (m^3 of airspace)/pig increased, the concentration of bacteria in the airspace decreased and the growth rate of the pigs increased significantly. I hypothesise that airborne bacteria trigger an immunological challenge which redirects metabolic activity that would otherwise contribute to growth and skeletal muscle accretion.

There is evidence that shed design and management can affect air quality and, consequently, growth rate of pigs. The results indicate that improving ventilation through widening ridge vents, leaving floors to dry before restocking pens, increasing pit depth to ≥400 mm, and flushing pits with fresh water all have a positive effect on air quality and growth rate. It is clear that facilities need to be managed as an all-in/all-out (AIAO) system as this enables farmers to maximise hygiene by thoroughly cleaning pens between batches, which is likely to improve air quality. Other important management and husbandry factors include adhering to stocking density (m^3 airspace/pig) and stocking rate (pigs/m^2 floorspace) recommendations, especially in naturally ventilated buildings. The shape and dimensions of the shed, the ventilation and heating system used, and the effluent management system are also important. Maintaining good air quality is essential for pig health, growth, and welfare, as well as those working with pigs.

Statement of originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Signed: _____

Timothy Wayne Murphy
December, 2011

Dedication

For my darling wife Lesley

.....Forever

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Abbreviations used in this thesis

Age-segregated rearing	**ASR**
Allophycocyanin	**APC**
All-in/all-out	**AIAO**
Alpha haemolytic cocci	**AHC**
Average daily gain	**ADG**
Bacteria	**Bac**
Batch Farrowing	**BF**
Beta-glucan	**β-1,3-glucan**
Bronchial Associated Lymphoid Tissue	**BALT**
Bronchoalveolar lavage	**BAL**
Bronchoalveolar lavage fluid	**BALF**
Cell Mediated Immunity	**CMI**
Colony forming unit	**cfu**
Degrees Celsius	**^{O}C**
Endotoxin Unit	**EU**
Ethylenediaminetetraacetic acid	**EDTA**
Feed conversion ratio	**FCR**
Fluorescein isothiocyanate	**FITC**
Forced expiratory volume-in-one-second	**FEV_1**
Forced expiratory flow rate at 25-75% of the FVC	**FEF_{25-75}**
Forced vital capacity	**FVC**
Gram	**g**
Hour	**h**
Hygiene air quality	**HAQ**
Immunoglobulin	**Ig**
Inspirable particles	**TD**
Insulin-like growth factor 1	**IGF-1**
Interleukin-1	**IL-1**
Intracerebroventricularly	**ip**
Intraperitoneally	**icv**
Kilogram	**kg**

Litre	**l**
Litre per minute	**l/min**
Metabolisable energy	**ME**
Micron	**μm**
Milligram	**mg**
Millilitre	**ml**
Minute	**min**
Nanogram	**ng**
Nanomole	**nMol**
Natural killer	**NK**
Occupational health and safety	**OH&S**
Parts per million	**ppm**
Peridinin Chlorophyll Protein	**PerCP**
Peripheral blood mononuclear cells	**PBMC**
Phosphate buffered saline	**PBS**
Phycoerythrin	**PE**
Red blood cells	**RBC**
Relative Humidity	**RH**
Respirable particles	**RP**
Revolutions per minute	**rpm**
Second	**sec**
Segregated early weaning	**SEW**
Standard error of the mean	**SEM**
Standard deviation	**SD**
Stocking density	**StD (m^3 airspace/pig)**
Stocking rate	**pigs/m^2 floorspace**
Streptavidin-Cy-Chrome	**CyC**
Total dust	**TD**
Viridans-group streptococci	**VGS**
Voluntary feed intake	**VFI**
White blood cell	**WBC**

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“Homer: Wait a minute wait a minute wait a minute. Lisa honey, are you saying you're *never* going to eat any animal again? What about bacon?

Lisa: No.

Homer: Ham?

Lisa: No.

Homer: Pork chops?

Lisa: Dad! Those all come from the same animal!

Homer: [*Chuckles*] Yeah, right Lisa. A wonderful, magical animal”

The 7th Season of the Simpson’s – Lisa the Vegetarian (3F03)

www.snpp.com/episodes/3F03.html

1

Introduction

Global economic and agricultural policies have driven agricultural enterprises in most western countries to become larger, more intensive and more specialised (Donham, 2000), with greater capital investment and less labour input. Intensive housing systems were first applied to poultry production in the 1950's in the United States of America, with intensive swine production appearing in Europe in the early 1960's and in North America in the late 1960's and early 1970's (Donham, 1995). Intensive swine and poultry production facilities have also begun to appear in developing countries, including Mexico, South America and the Pacific Rim countries including Taiwan and the Philippines (Donham, 1995).

Intensification has coincided with increased consumption and decreasing retail prices relative to income, particularly in the developed world. As a consequence, pork and its processed products is one of the most popular meats consumed in the world today. Globally, more than 83 million mega tonnes are produced each year from more than one thousand million pigs slaughtered (Department of Agriculture, Fisheries and Forestry, 2009).

The global changes to the pork industry have been mirrored in Australia. Australia is ranked 25th in the world for pig meat production with 4,616,700 pigs slaughtered annually, producing 338,735 tonnes of pigmeat (Australian Bureau of Statistics, 2011). The gross value of the Australian pork industry during the 2009/2010 financial year was $902.8 million (Australian Bureau of Statistics, 2011a). Australia has 2,456 pig farms (Australian Bureau of Statistics, 2011b) with a total herd size of 2,289,292 pigs. In

Australia, pig producers are located throughout all states and are generally aligned with grain growing regions. In Australia, the average finisher pig sale liveweight was 93.18 kg, with an average age-at-sale of 153.37 days. The average growth rate was 603.48 g/d with an average feed conversion ratio (feed eaten:weight gain) of 3.19 (Australian Pork Limited, 2011). The pig industry is a high cost, high technology, intensive industry with narrow margins, which must achieve high levels of efficiency if adequate returns on investment are to be maintained (Hope, 1990). It is also a rapidly changing industry, noted for innovation and preparedness to adopt new technologies. One of the main aims of a piggery is to have a cost-efficient production without compromising the welfare requirements of the pigs and those working with pigs.

In Australia, more than 90% of the existing intensive pig sheds are naturally ventilated because of the mild winters (Wang, 2003). The flooring of these sheds is usually partially slatted (20 – 30% of total pen floorspace) which allows water, urine and faeces to fall through into under-floor channels or pits. These pits are regularly flushed, or drained to remove effluent from the sheds.

The growth rate of pigs raised under commercial conditions is well below their potential when housed in an ideal environment (Black and Carr, 1993; Chapple, 1993; Williams *et al*., 1994; Cargill *et al*., 2000; O'Doherty and McKeon, 2000; Alcantara *et al*., 2008). This so-called 'growth gap' has a significant impact on the potential profitability of a pig enterprise. Many factors within a commercial environment potentially contribute to the depression in feed intake, growth rate and efficiency of feed use, and to the tendency for

increased fatness of the carcasses (Edmonds *et al*., 1998; Nyachoti *et al*., 2004). These factors include the stocking rate (pigs/m^2 floorspace), stocking density (m^3 airspace/pig), prevalence of disease, the temperature and humidity inside the shed and the quality of the air including gases, dust and bacterial load.

The environment created in modern pig housing facilities has been shown to have a major influence on the growth rate, immune function, physiology and welfare of the pig, as well as the health of stockpersons involved in pig production (Gerber *et al*., 1991; Hartung and Phillips, 1994; Donham, 1995; Cargill and Hartung, 2001; Le Floc'h *et al*., 2006). In particular, air quality has been a major concern for production specialists, veterinarians and pig producers in many countries, including Australia, for at least three decades (Donham *et al*., 1977; Cargill and Skirrow, 1997, Donham, 2000). Added to these concerns is the association between airborne particle emissions and odour emissions from swine confinement buildings to the surrounding community (Cargill and Skirrow, 1997; Nimmermark, 2004; Radon *et al*., 2004; Wathes *et al*., 2004; Duan *et al*., 2009).

The major contributors to poor air quality are airborne particles (including bacteria) and ammonia. In Australia, viridans-group streptococci (VGS) are representative of the streptococci bacteria that occur in pig sheds (Cargill and Skirrow, 1997; Done *et al*., 2005). Airborne particles may also act as vectors in the spread of other infections between buildings housing livestock, and it has been suggested that buildings need to be

at least 100 to 150 metres apart to prevent dust-driven disease spread (Collins and Algers, 1986; Duan *et al*., 2009).

Other problems associated with airborne dust include equipment failure, especially when very fine dust particles contaminate electrical equipment such as fans, and inflatable polythene ducts used for ventilation (Carpenter, 1986). Gases, such as ammonia, may hasten the corrosion of equipment and building structures, increasing the cost of maintenance.

Poor air quality may also affect the pigs' immune function. The influence of immune activation on growth has been documented in the poultry industry (Kelley *et al*., 1987, Klasing and Barnes, 1988). In chickens, immune stimulation reduced body weight gain and gain:feed ratios by 17.1 and 17.0%, respectively, compared with those of pair-fed controls. However, the magnitude of the differences in requirements for both methionine and lysine could not be evaluated because a limited number of amino acid levels were fed.

A number of studies (Knowles *et al*., 1997; von Borell *et al*., 2007; Le Floc'h *et al*., 2009) have shown that poor sanitary conditions in pig sheds are associated with the induction of inflammatory responses, and that inflammatory activation leads to slower growth. In part, the slower growth arises from a reduced appetite (Escobar *et al*., 2004; Renaudeau, 2009), but Sandberg *et al*., (2007) concluded that the immune response itself has a nutrient demand. The mediators, proinflammatory cytokines such as

interleukin-1 beta released by activated mononuclear immunocytes (Johnson, 1998), have also been shown to initiate catabolism of skeletal muscle (Dionissopoulos *et al*., 2006). Indicators of such a cellular immune response are acid glycoproteins (Sauber *et al*., 1999; Greiner *et al*., 2000; Grellner *et al*., 2002) and the CD4+:CD8+ ratio of T lymphocytes (Davis *et al*., 2004; Clapperton *et al*., 2005).

Specific factors, called cytokines, are produced and secreted by the pig's white blood cells as a defence mechanism in response to endotoxins. Cytokines suppress the secretion of the significant growth promoting hormones, affect blood glucose homeostasis, increase protein oxidation, increase muscle proteolysis and alter metabolic processes (Almond *et al*., 1996; Johnson, 1998). Thus, immunological challenge redirects metabolic activity that would otherwise potentially contribute to growth and skeletal muscle accretion in order to enhance metabolic processes that support the immune response. The alteration in metabolism involves a decrease in plasma insulin-like growth factor-1 (IGF-1) concentrations. It is probably for this reason that dietary manipulation fails to improve pig growth after immunological challenge (Black *et al*., 2001).

In order to further study the effects of bacterial aerosols and ammonia gas on feed intake and growth rate, and the physiology and immune function of the respiratory system, in pigs, a series of objectives were developed. These were to investigate the effects of;

- ammonia and alpha haemolytic cocci (AHC) including viridans-group streptococci (VGS) on the feed intake, immune function and physiology of the respiratory tract in pigs.
- stocking density on air quality parameters (ammonia, dust and bacteria) and growth rate in pigs;
- improving shed design and management on air quality parameters (ammonia, dust and bacteria) and growth rate in pigs;
- test and validate a number of strategies for reducing selected air pollutants in pig buildings including slat type, pit depth, time taken to re-stock pens and the use of fresh *vs* recycled water.

There were three elements to this project. These were (1) a trial of the effect of ammonia and bacteria on feed intake, immune function and physiology of the pig; (2) a trial of the effects of stocking density on air quality parameters and growth rate and (3) a series of case studies related to; the effect of ridge vent and stocking density on air quality parameters and growth rate; the effect of wet versus dry floors prior to re-stocking on air quality parameters and growth rate; the effect of pit depth and slate type on air quality parameters; and the effect of fresh, versus recycled, water on air quality parameters.

The null hypotheses to be investigated were;

1. Ammonia and alpha haemolytic cocci (AHC) including viridans-group streptococci (VGS) have no effect on feed intake, immune function or physiology of the respiratory tract of pigs.

2. Stocking density has no effect on air quality parameters (ammonia, dust and bacteria) and the growth rate of pigs.

Having determined that air quality parameters (ammonia and bacteria, and stocking density) has an effect on immune stimulation and growth rate, a series of on-farm case studies were performed to investigate the effects of improving shed design on air quality and growth rate in pigs.

2

Literature review

2.1 Overview of Chapter

In this chapter an overview of the relevant research and literature is discussed. The nature of pig production in Australia is outlined. A summary of the airborne pollutants affecting the health and growth rate of pigs, and the health of humans working with pigs is given, namely gases, including ammonia, and particulate matter, including bacteria and endotoxins. In addition, occupational diseases experienced by piggery workers including the acute and chronic symptoms are identified. Furthermore, control measures and best practices that exist within the pig industry to improve air quality and their possible implications on pig and human health are discussed.

2.2 Pig production systems in Australia

Until recently, the majority of pigs in Australia were housed in conventional farrow-to-finish operations called 'continuous flow' (CF), with the breeding and growing herd housed on the one site. Most of these facilities have open pen divisions which allow substantial contact between pigs in adjacent pens, so that each shed can be regarded as a large pen housing several hundred pigs varying in age by as much as 13 weeks (Cargill *et al*., 1997). Such practices encourage the spread of diseases from the sow to the litter and from pig-to-pig throughout the growing phase. In particular the exposure of younger pigs to disease is increased by them being mixed in the same airspace with older, infected pigs (Clark *et al*., 1991). Batching pigs by age group and managing the facilities all-in/all-out (AIAO), overcomes many of the problems associated with conventional continuous flow (CF) production (Dial *et al*., 1992; Holtkamp, 1995). AIAO facilities may be filled over a period of two weeks, and emptied over a similar

period, but all pigs must be moved out, and all pens cleaned thoroughly, before the next batch of pigs moves in (Cargill *et al*., 1997). AIAO provides the opportunity to house pigs in a clean environment, which reduces the level of immunological stress on the pig and the productivity losses associated with it, as well as improving working conditions for staff (Holtkamp, 1995). Age-segregated rearing (ASR) describes a management system where pigs are reared together in batches with no more than 2 weeks separating the oldest and youngest pig in the group. By definition, ASR means that pigs are managed on an AIAO basis.

2.3 Airborne pollutants

Air quality is a characterisation of the air content compared to its normal composition under clean conditions (ASHRAE, 1999). Air quality is an assessment of how many contaminants, such as gases and particulate matter, are present in addition to the various gases constituting normal clean air. The more contaminants present in the air, the lower the air quality.

The important airborne pollutants affecting the health and growth rate of pigs, and the health of humans working with pigs, have been identified as gases, dust and bacteria. Today, air contaminants in pig buildings can broadly be categorised into two categories; gases and particulate matter. Gases are predominantly produced directly by the pigs and their excreta. The primary gases affecting pig and human health are ammonia (NH_3), carbon dioxide (CO_2), hydrogen sulphide (H_2S) and carbon monoxide (CO) (Donham *et al*., 1982; Cole *et al*., 2000). Particulate matter is composed of faeces, feed

components, skin cells, and the products of microbial action on faeces and feed (Table 2.1). Components of feed include plant proteins, starches and carbohydrates; feed additives such as vitamins, minerals, amino acids and other supplements; and antibiotics. Bioaerosols, particles of biological origin that are suspended in air, are a major component of the particulate matter in pig buildings. Bioaerosols include bacteria, fungi, fungal and bacterial spores, viruses, mammalian cell debris, products of microorganisms, pollens, and aeroallergens. Bacterial and fungal bioaerosols may be of infectious, or non-infectious species. Bacterial products or components exist as bioaerosols and include endotoxins and peptidoglycans. Fungal products or components include mycotoxins and glucans (Heederik *et al*., 2002).

Table 2.1: Potentially hazardous agents found in bioaerosols from livestock buildings (Donham, 1989).

NOTE:
This table is included on page 35 of the print copy of the thesis held in the University of Adelaide Library.

The current safe maximum exposure limits recommended in Australia for selected air pollutants in pig sheds are shown in Table 2.2.

Table 2.2: Current safe maximum exposure limits recommended in Australia (Cargill and Skirrow, 1997; Cargill *et al*., 2002).

Pollutant	**Maximum Safe Concentration**
Ammonia	10ppm
Inhalable particles	2.4 mg/m^3
Respirable particles	0.23 mg/m^3
Respirable endotoxins	50 EU/m^3
Total airborne bacteria	1.0 x 10^5 cfu/m^3

The human and pig maximum levels for various air pollutants found in pig sheds are shown in Table 2.3.

Table 2.3 – Recommended human and pig exposure levels for various air pollutants found in pig sheds (Donham, 1995; Banhazi *et al*., 2008).

Pollutant	**Human health**	**Swine health**
Ammonia (ppm)	7.0	11.0
Carbon dioxide (ppm)	1,540	1,540
Total dust (mg/m^3)	2.4	3.7
Respirable dust (mg/m^3)	0.23	0.23
Endotoxin (g/m^3)	0.08	0.15
Total bacteria (cfu/m^3)[a]	4.3 x 10^5	4.3 x 10^5
Total bacteria (cfu/m^3)[b]	1.0 x 10^5	1.0 x 10^5

[a](Donham, 1995), [b](Banhazi *et al*., 2008)

The standard of surface and air hygiene within pig buildings is dependent on a series of complex interactions between building design and animal management and behaviour (Gustafsson, 1994; Banhazi *et al*., 2000; Cargill *et al*., 2002). Hygiene and air quality are influenced by several building characteristics, including the shape and dimensions of the building, the ventilation and heating system used and the effluent management system (Cargill and Banhazi, 2002). Shed environmental factors include the level of cleaning and disinfection, the state of the pen floors, watering and feeding systems, and the quality of water used for cleaning and effluent removal. Husbandry factors include stocking rate (pigs/m^2 floorspace) and stocking density (m^3 airspace/pig), and shed population size (Skirrow *et al*., 1995)

The effects of these pollutants on pig health and production will vary depending on the mixture and concentrations of the pollutants present. Hence, each day, intensively housed pigs will inhale a number of gases and millions of bioaerosols, some of which are potentially pathogenic. Depending on the size of these particles, they will be deposited at various levels of the respiratory tract.

2.4 Gases

The primary gases affecting the health of pigs and workers include ammonia, carbon dioxide, hydrogen sulphide and carbon monoxide (Donham *et al*., 1977).

2.4.1 *Ammonia*

In terms of air quality, ammonia is the most common gas present in pig sheds that affects the health and welfare of both pigs and humans (Payne 1994; Cargill and Skirrow, 1997; Donham *et al*., 2002). Ammonia is highly water soluble, and reacts with moist mucosal surfaces of the eyes, respiratory tracts, and skin to form a corrosive alkaline solution (ammonium hydroxide). In this state ammonia has the capacity to cause liquefaction necrosis (Swotinsky, 1990; Groot Koerkamp *et al*., 1998).

Ammonia concentration within pig sheds is 250 to 750 times higher in summer and greater than 1,500-fold in winter and spring compared to atmospheric ammonia concentrations (Subramanian *et al*., 1996). Concentrations on most farms in Australia range from 3 to 20 parts per million (ppm) (Skirrow *et al*., 1995; Banhazi *et al*., 2000). The maximum acceptable level in pig sheds for ammonia has been set as low as 10 ppm with the target level set at less than 7 ppm (Payne, 1994; Banhazi and Cargill, 1996). The rationale for the suggested levels of ammonia are based mainly on exposure-response studies in swine workers (Donham *et al*., 1989; Donham *et al*., 1995; Reynolds *et al*., 1996; Donham *et al*., 2002).

Even in well ventilated sheds where ammonia concentrations are less than 5 ppm at pig height (~50cm above the slats), the levels of ammonia rising through the slats can be as high as 15 to 20 ppm (Cargill and Skirrow, 1997). Consequently, the pig will breathe in large quantities of ammonia when it is recumbent.

2.4.1.1 Source of ammonia

The main sources of ammonia emissions are the metabolic processes of degrading urea, which is excreted via urine. Urea is converted into ammonia and carbon dioxide by the enzyme urease, present in faeces. The most important factors affecting this process are the urinary urea concentration, pH and effluent temperature. Raising the pH of effluent from 7.0 to 7.3 and 7.3 to 7.6 increases ammonia evaporation by approximately 20% and 100%, respectively (Pedersen, 1993). Temperature and wind velocity also have a similar, but less dramatic, effect on evaporation. The depth of the pit and the distance between the surface of the slurry and the slats, both affect air movement over the surface, as well as the temperature of the slurry and evaporation of ammonia.

Because pig buildings are ventilated for temperature control, the concentration of ammonia tends to peak in the early morning, prior to opening sheds (Cargill *et al*., 1997). As concentrations are highest at slat level, animals in sheds with totally slatted floors are exposed to maximum concentrations whenever they are recumbent (Gerber *et al*., 1991; Aarnink and Swierstra, 1995; Cargill and Banhazi, 2002). By comparison, with partially slatted floors, pigs lying on a clean solid floor receive minimum exposure. However, a study by Lee *et al*., (2005), reported that if floors are covered with faeces and urine, ammonia levels will be higher (13 vs 6 ppm). Concentrations of ammonia vary in deep litter systems, and are highest when animals or humans disturb the litter (Banhazi *et al*., 2000).

2.4.1.2 Effects of ammonia on pigs

In a number of studies where pigs were exposed for short periods to concentrations of ammonia above 35 ppm, inflammatory changes in the wall of the respiratory tract, as well as reduced bacterial clearance from lungs, were evident, as well as reduction in growth rate of 12% (Drummond *et al*., 1978; Johannsen *et al*., 1987). Pigs exposed to ammonia also harboured more bacteria (non pathogenic Escherichia coli) in their lungs than pigs in an ammonia-free atmosphere, and the response appeared to be dose dependent. The clearance of inhaled bacteria was also inhibited when pigs were subjected to cold temperatures of $6^{O}C$, with younger pigs harbouring more viable bacteria in their lungs than older pigs (Drummond *et al*., 1978). A study by von Borell *et al*., (2007) demonstrated elevated monocyte (52%), lymphocyte (45%) and neutrophil (2%) counts in weaner pigs exposed to ammonia at a concentration of 35 ppm. In another study, no pathological effects were noted (Curtis *et al*., 1975). There is also no clear consensus on the physiological effects of lower concentrations of ammonia, but the gas may also interact with other biological agents to enhance inflammatory changes (Gustin *et al*., 1994).

A mild chronic sub-epithelial infiltrate has also been observed in sections of trachea taken from pigs exposed to ammonia (Stombaugh *et al*., 1969), along with loss of cilia, thickened epithelia and decreased numbers of goblet cells in the trachea and turbinates (Doig and Willoughby, 1971). Ciliary loss, with decreased numbers of goblet cells, has also been observed in turkeys and lambs exposed to ammonia (Drummond *et al*., 1976).

The effects of ammonia on growth appear to be variable. For example, in pigs exposed for four weeks to filtered air containing 0, 50, 100 and 150 ppm ammonia, growth rates were reduced by 0, 12, 30 and 29% respectively (Drummond *et al*., 1980). Growth rate suppression following exposure to ammonia was also observed by Stombaugh *et al*, (1969), but not by Doig and Willoughby (1971) or Curtis *et al*, (1975). The studies by Drummond *et al*, (1980) and Stombaugh *et al*, (1969) however, did not account for the amount of feed eaten, or the feed wasted, hence an accurate measure of growth rate was not obtained. The inconsistent observations suggest that unless standardised experimental designs are used, which ensure pigs are not exposed to other pollutants, such as bacteria, the results may be invalid or difficult to interpret, hence it may not be ammonia *per se* that affects growth rate.

Pig activity (as determined by whether animals were standing, walking, sitting or lying during a 5 minute period) also appears to be affected by exposure to ammonia. When pigs were exposed to 100 or 150 ppm of aerial ammonia, they were lethargic early in the study in comparison to control animals, or pigs exposed to only 50 ppm, but became more active as the study continued (Drummond *et al*., 1980).

The clinical signs attributed to ammonia exposure have also been well documented (Stombaugh *et al*., 1969; Drummond *et al.,* 1980; Donham *et al*., 1989). They include coughing, sneezing, salivation, loss of appetite and excessive lachrymal secretions. Increased lachrymal secretions was associated with the development of black ‘patches’ extending from the corner of the eye (Drummond *et al.,* 1980), and the size of these

patches appeared to be directly proportional to the ammonia concentration. However, in another study (Doig and Willougby, 1971), neither coughing nor sneezing was evident in pigs exposed to levels of ammonia up to 150 ppm.

When gilts were exposed to a low (4 to 12 ppm) or moderate concentration (26 to 45 ppm) of ammonia, a difference in growth rate was evident during the first 2 weeks (Diekman *et al.,* 1993). However after 4 and 6 weeks exposure, no significant differences could be demonstrated. It should also be noted that pigs in this study were exposed to a range of other pollutants as well, hence the effects were not due to ammonia alone.

The effect of ammonia on reproductive performance is inconsistent. Although delayed puberty and an increased susceptibility to certain respiratory diseases have been reported (Diekman *et al*., 1994), another study failed to demonstrate an effect on the onset of puberty in gilts (Diekman *et al*., 1993). In the latter study, growth of ovarian, uterine and adrenal tissues was not affected by 6 weeks exposure to a moderate concentration of ammonia. Also, exposing gilts to 36 ppm ammonia (mean aerial concentration) did not alter the age of pubertal oestrus, even though gilts were lighter in weight at the onset of puberty.

Studies with other species have tended to produce comparable results to those recorded with pigs. For example, exposure of chickens to aerial ammonia at concentrations of 75 to 100 ppm depressed growth, but not lower concentrations, such as 50 ppm (Charles

and Payne, 1966). On the other hand, lambs appear to be more sensitive to aerial ammonia than pigs, as exposure to 75ppm for 28 days reduced body weight gain by about one third (Drummond *et al*., 1976).

A number of studies have investigated the potential synergistic effects of ammonia and other airborne pollutants, such as respirable dust and endotoxins, resulting in inflammatory changes (Gustin *et al*., 1994; Urbain *et al*., 1996b; Done *et al*., 2005). For example, although nebulisation with endotoxin alone had no direct effect on the nasal mucosa of healthy pigs, animals nebulized with endotoxin after exposure to ammonia (50 ppm) had increased neutrophil counts and elevated albumin concentrations in nasal lavage (NAL). In contrast, in pigs exposed to various concentrations of ammonia gas (0.6 – 37 ppm) and dust (mixture of feed, barley straw and faeces; 1.2 to 9.9 mg/m^3) for 5 weeks there were no significant effects on pig health as determined by signs of rhinitis, pleurisy, pleuropneumonia-like lesions and abscesses (Done *et al*., 2005). These conflicting results may be due to differences in the experimental protocols, for example, the immune status of the pigs, dose, timing and method of inoculation of the pathogen(s), and the nature of the dust.

2.4.1.3 Effects of ammonia on humans

The acute irritant effects of ammonia have not been well correlated with levels of exposure in humans (Swotinsky, 1990) and relatively little is known about the health effects, if any, of long-term inhalation exposure.

A number of earlier experimental studies have reported the effects of short-term ammonia exposure in humans (Silverman *et al*., 1949; MacEwen *et al*., 1970; Keplinger *et al*., 1973; Verberk, 1977; McLean, 1979). In these studies the ammonia concentrations ranged from 30 ppm to 500 ppm, with exposure ranging from 30 seconds to 2 hours. Concentrations less than 50 ppm, caused nasal dryness, while at concentrations greater than 50 ppm, subjects reported eye, nose and throat irritation. There were no significant differences in haematologic and spiromentric tests, pre- and post-exposure.

In a study by Donham *et al*., (1977) of 21 workers, spirometry measurements were taken before and after 4-hour shifts in swine confinement buildings. There were significant differences in forced vital capacity – the total amount of air the subject can expel during a forced expiration (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory flow rate at 25-75% of the FVC (FEF_{25-75}) in the workers. However, the relative contribution of ammonia to these changes is unknown, as the exposure would have included a number of various gases and particulate matter, including bioaerosols.

There is little information about the effects of long-term exposure to ammonia. In a small study by Ferguson *et al*., (1977) six nonacclimated adults were exposed to 3 concentrations of ammonia; 25, 50, and 100 ppm for 2 to 6 hours per day, 5 days per week, for 6 weeks. Mild eye and nasal irritation were reported in response to exposure to 100 ppm, but not at 25 ppm, and the symptoms resolved over time. A study by

Holness *et al*., (1989) compared the long-term effects of ammonia exposure on pulmonary function, eye, skin, and respiratory symptoms in 52 soda ash workers and 31 control subjects. The soda ash workers were exposed on average for 12.2 years to 9.2 ppm ammonia, while control subjects were exposed to 0.3 ppm ammonia. There were no differences in any of the endpoints (respiratory symptoms, sense of smell, baseline lung function, or change in lung function over a work shift at the beginning and end of a work week) between the two groups.

Many studies have evaluated the potential cross-shift declines in pulmonary function tests, such as FEV_1 and FEF_{25-75} in poultry workers, who work in environments with high concentrations of dust, endotoxin, ammonia and bacteria (Thelin *et al*., 1984; Morris *et al*., 1991; Reynolds *et al*., 1993; Zuskin *et al*., 1995). However, there has been little research investigating the potential synergistic effects of ammonia and other airborne pollutants, such as respirable dust and endotoxins resulting in inflammatory changes and pulmonary function in humans. A study by Donham *et al*, (2002) investigated the pulmonary function of 257 workers from the poultry industry, as well as airborne pollutants (total and respirable dust, ammonia, endotoxin and CO_2) from their work environment. The synergy between ammonia levels and airborne dust explained up to 43% and 63% of the decline in pulmonary function (FEV_1 and FEF_{25-75}) over the work shift. The combination of total dust and ammonia in poultry housing was found to have greater than additive health effects on workers. These studies have demonstrated that intensive farms need to develop control measures to reduce both dust (including bacteria and endotoxins) and ammonia. The studies also confirmed that

exposure limits for combined pollutants should be lower than those recommended for individual pollutants (Donham *et al*., 2002).

2.4.1.4 Reduction and control of ammonia

Historically, there have been three main approaches to reduce the levels of ammonia in pig housing facilities. These are, (1) changes to the diet, (2) improved effluent disposal and (3) improved ventilation systems.

Diets can be modified by lowering protein levels and changing amino acid balance to reduce nitrogen excretion. Feed additives such as yucca extracts, which bind ammonia and enzymes, have been shown to cause a small, non-significant reduction in ammonia emissions from pig effluent (Cole, 1994; Colina *et al*., 2001), however, have not been widely adopted by Australian piggeries.

There are a variety of aerobic and anaerobic digestion processes, as well as slurry activators, which can be used to change the chemical properties of slurry and reduce ammonia emissions. Ammonia is water soluble and this has led to two common practices in waste management to reduce ammonia emissions. The first is the use of a deep pit, which allows urine to mix with water and faeces and be covered with a layer of water to reduce ammonia evaporation. Emptying the pits less frequently has also been shown to reduce ammonia emissions (Cargill and Skirrow, 1997). The other approach is to remove faeces frequently either using a scraper followed by flushing or

flushing effluent channels with large quantities of water (Groenestein, 1994). The scraper method has not been widely adopted in Australian piggeries.

Ammonia gas is less dense than air and can, accordingly, be exhausted from sheds. However, if increasing the ventilation results in increased air movement over the surface of the pits, it will also increase ammonia production. Ventilation systems that exhaust air from above the floor to below the floor will reduce ammonia concentrations at pig level (Van't Klooster *et al.*, 1993). Under-slat ventilation systems can form a crust layer on top of the slurry, allowing vermin to move along it (Demmers, 2002 pers comm.). Increasing the ventilation rate from 20% to 60% has been shown to reduce ammonia levels by 57% in pig sheds (Kim *et al*., 2007), however, there was no significant reduction in airborne microorganisms or total dust. These results demonstrate that ventilation of pig buildings has a complex effect on the concentration of airborne pollutants. Ventilation systems are designed to control the thermal environment and facilitate the removal and transportation of airborne pollutants outside the building via exhausted air. It is likely that ventilation rate has little effect on the gradient of particulates, especially total dust, due to gravity generated by their size and weight. The finding that the concentrations of total airborne microorganisms were not also significantly different among the ventilation rates can be supported by the fact that airborne microorganisms are easily adsorbed on the surface of dust particles.

2.4.2 *Carbon dioxide*

Carbon dioxide gas is more dense than air and, therefore, tends to accumulate at pig level (Banhazi and Cargill, 1999). Ambient air contains 300-400 ppm of carbon dioxide gas and carbon dioxide concentration is a good measure of the ventilation rate. It can also be used as an indicator of the general level of ventilation inside livestock buildings (Barber *et al*., 1993). Recommended levels for carbon dioxide vary, but a good target is less than 1500 ppm with 3000 ppm being the absolute maximum (Pointon *et al*., 1995).

2.4.2.1 Source of carbon dioxide

Pigs are responsible for producing most of the carbon dioxide present in sheds, and levels will vary according to stocking and ventilation rates, as well as pig activity.

2.4.2.2 Effects of carbon dioxide on pigs and humans

Carbon dioxide at very high levels (20,000 ppmhas been shown to cause tiredness, lethargy and death in humans (Donham, 1995). However, such levels are rarely found in piggery buildings. Reduced growth rate and increased prevalence of respiratory disease in pigs have been associated with levels of carbon dioxide above 1500 ppm (Donham *et al*., 1989). However, many of the sheds in this study also had high levels of ammonia, which may have affected the results observed in growth rate and prevalence of respiratory disease.

2.4.2.3 Reduction and control of carbon dioxide

High levels of pig activity and high stocking rates are directly related to carbon dioxide concentration. Increasing ventilation rates and reducing the number of pigs per cubic metre of airspace will lower carbon dioxide levels (Gerber *et al*., 1991). Increasing air exchange by increasing ventilation rates for short periods is considered useful as it will clear excess carbon dioxide and ammonia from the shed without a long term drop in temperature (Cargill and Skirrow, 1997). It has been reported that carbon dioxide may be a good indicator for other airborne pollutants (Donham, 1991). In this study, low levels of carbon dioxide (from good ventilation) resulted in lower concentrations of endotoxin, microbes, and ammonia, but not total dust concentration.

2.4.3 *Hydrogen sulphide*

Hydrogen sulphide is a highly toxic, colourless, flammable gas with an offensive odour resembling that of rotten eggs. Hydrogen sulphide is not present in great quantities in pig sheds in Australia, due to the nature of effluent removal systems employed. Hydrogen sulphide is an extremely toxic gas and has been responsible for several human deaths in North America and Europe, as well as mortalities in pigs (Donham, 1995; Banhazi and Cargill, 1996). Recommended levels in pig sheds are less than 5 ppm.

2.4.3.1 Source of hydrogen sulphide

Hydrogen sulphide is a product of the anaerobic decomposition or organic (primarily manure) material so that anaerobic liquid manure storage pits are the primary source. Large quantities of the gas (200 - 800 ppm) can be released when the slurry is agitated.

2.4.3.2 Effects of hydrogen sulphide on pigs and humans

Studies in Western Europe and North America have demonstrated that pigs continually exposed to hydrogen sulphide concentrations of 20 ppm had a reduced feed intake, increased stress markers and a fear of light. Levels of 200 ppm caused pulmonary oedema, breathing problems and death (Gerber *et al.*, 1991). In humans, hydrogen sulphide levels between 10 and 20 ppm cause eye and upper respiratory tract irritation while levels between 50 and 100 ppm cause vomiting, nausea and diarrhoea, and levels greater than 500 ppm have the potential to cause unconsciousness and death (Donham, 1995).

2.4.3.3 Reduction and control of hydrogen sulphide

Care needs to be taken when slurry pits are emptied or agitated, especially if they are located in a confined area (Donham, 1995; Jacobson *et al*., 2007). Ideally, slurry or manure storage pits should be constructed outside the pig building.

2.4.4 *Carbon monoxide*

Carbon monoxide is a highly poisonous colourless and odourless gas. It is only a problem where combustible fuel is used for heating (Gerber *et al*., 1991). Carbon

monoxide is generally not an issue in Australian piggeries as fossil fuel burners are not used.

2.4.4.1 Source of carbon monoxide

The main source of carbon monoxide is from gas burners, used to heat piggeries in cold climates (Gerber *et al*., 1991).

2.4.4.2 Effects of carbon monoxide in pigs and humans

Carbon monoxide concentrations of 150 ppm have been shown to induce porcine abortion, increase the incidence of stillborn pigs, and reduce growth rate among young pigs (Gerber *et al*., 1988). Levels ranging from 200 to 300 ppm adversely affect rate of gain and feed efficiency (Morris *et al.*, 1985).

Barker *et al.,* (1996) reported that swine workers exposed to carbon monoxide at a concentration of 50 ppm for eight hours experienced fatigue and headaches. Workers exposed to a concentration of 500 ppm for three hours experienced impaired judgement, chronic headaches and nausea. Carbon monoxide at 15 ppm for 10 hours affected the central nervous system (Perkins, 1974). Manahan (1975) reported that continuous exposure to carbon monoxide levels of 7 to 10 ppm causes impaired judgement and visual perception in humans; 100 ppm caused headache, dizziness and weariness; 250 ppm caused loss of consciousness; 750 ppm caused death after several hours; and 1000 ppm caused rapid death.

2.4.4.3 Reduction and control of carbon monoxide

In piggeries where unit space heaters that use combustible fuel are used, it is important that they are checked and maintained regularly. Carbon monoxide levels will increase with incomplete combustion of fuels such as wood, gas, coal, kerosene, oil or diesel (Gerber *et al.*, 1991).

2.5 Particulate matter – dust

Traditionally, the term 'dust' was used to describe the complex mixture of non-gaseous pollutants in the airspace. Dust was specified in terms of its physical, chemical and microbial properties (Demmers *et al*., 2003). Physical properties include the concentration of particles by number and mass and the size distribution of particles by number and mass. Chemical properties include toxins and allergens. Microbiological properties include the number of viable and non-viable bacteria, viruses and fungi and the endotoxin content.

Today, the term used to describe the complex mixture of non-gaseous pollutants in the airspace is 'particulate matter'. Particulate matter is not a single pollutant, but a mixture of many types of pollutants. The term particulate matter is often used to refer to fine solid or liquid particles suspended in a gaseous medium (Zhang 2004). The term dust now refers only to solid particles of matter formed by mechanical fracture (ie. crushing) of a parental material, which sediment under gravity forces (Zhang, 2004). Particulate matter can be defined as a complex mixture of suspended particles with different

physical, chemical and biological characteristics, which determine both its behaviour, as well as its environmental and health effects (EPA, 2004).

Particulate matter in pig buildings differs from other types of particles for three main reasons: (1) its concentrations are generally 10-100 times higher than in other indoor environments; (2) it is an odour and gas carrier; and (3) it is biologically active, generally containing a great variety of bacteria and microorganisms (Zhang, 2004).

The most relevant health hazards to humans of particulate matter inside pig buildings are related to respiratory diseases (Donham *et al*., 1984; Andersen *et al*., 2004). There is now evidence that particulate matter generated from pig buildings, when emitted from the ventilation exhausts, affects humans living nearby, leading to increased oxidative stress, and, as a consequence, increased prevalence of respiratory diseases, and mortality (Pope *et al*., 2002). Particulate matter outside pig buildings also represent a nuisance, caused by odorants adsorbed by airborne particles (Wathes *et al*., 2004).

Airborne particulate matter (traditionally called dust) is measured in mg/m^3 of air and classified in three ways, according to particle size, being inhalable, thoracic and respirable. Inhalable dust particles, which include thoracic and respirable dust particles, contain particulate matter up to 100 μm in diameter. These particles are inhaled through the nose and/or mouth during normal breathing. Some of these airborne particulates are trapped in the mucus of the nose and pharynx and prevented from travelling deeper into the lungs (Gordon, 1963). Thoracic dust particles, which include respirable dust

particles, contain particulate matter up to 10 μm (PM10) in diameter. These particles can penetrate the respiratory system beyond the larynx into the trachea and bronchi. Respirable dust particles contain particulate matter up to 4 μm in diameter (Zhang, 2004). Earlier studies referred to respirable dust particles as PM5 (Pedersen *et al*., 2000). Respirable dust particles can penetrate the smallest cavities of the lung, the alveoli, where gaseous exchange takes place.

In a study of 114 Australian pig sheds, which included all ages of pigs, the mean concentration of airborne inhalable particles and respirable particles were 1.74 mg/m^3 and 0.26 mg/m^3 respectively (Banhazi *et al.*, 2000). The maximum and minimum values were 10.07 mg/m^3 and 0.12 mg/m^3 for airborne inhalable particles and 2.13 mg/m^3 and 0.01 mg/m^3 for respirable particles. More importantly, in 41% of sheds, airborne inhalable particles were above the Australian recommended value (2.4 mg/m^3) and 58% of sheds recorded values for respirable particles above the Australian recommended value (0.23 mg/m^3) (Banhazi *et al*., 2008b). The current 'safe' maximum exposure limits recommended in Australia are 2.4 mg m^3 for inhalable particles and 0.23 mg m^3 for respirable particles (Banhazi *et al*., 2008b).

Levels of dust fluctuate from day to day, as well as during the day, with higher levels being associated with pig and human activity (Cargill and Banhazi, 1996; Banhazi and Cargill, 1997). Kim *et al*., (2005) observed higher dust levels between 2:00pm and 5:00pm, which was attributed to increased pig activity due to a cooler outdoor temperature. Dust levels have also been shown to fluctuate between seasons (Banhazi

et al., 2004) with levels that are higher in winter months (Robertson, 1993; Seedorf *et al*., 1998; Takai *et al*., 1998), with weaner pigs recording the highest levels (O'Shaughnessy *et al*., 2010). The highest levels are associated with feeding, sweeping, and moving and weighing pigs (Table 2.4). Low humidity, as well as very high and low levels of ventilation, result in increased airborne dust levels (Pedersen, 1989). The increase in dust levels from summer to winter occurs in both mechanically ventilated and automatically controlled, naturally ventilated sheds, and is associated with reduced ventilation rates in winter to maintain shed temperatures (Banhazi *et al*., 2004). The settling pattern of dust varies according to the particle size and weight, with the heavier particles being the first to settle and the last to be resuspended (Barber *et al*., 1991; Takai *et al*., 1998).

Table 2.4: Dust levels associated with work practices in a pig shed (Banhazi and Cargill, 1997; Banhazi *et al*., 2008b).

Work practice	Total dust (mg/m^3)
Hand feeding	20-25
Sweeping floors	20-25
Vacuuming floors	3-7
Dusting walls	25-30
Applying straw	6-14
Chopping straw	5-67
Weighing pigs	5-8
Recommended maximum level	2.4

The percentage of respirable particles compared to inhalable particles varies from building to building and the percentage of the respirable particle fraction has been

reported as ranging from 10% (Cargill and Skirrow, 1997), 30% (Donham *et al.,* 1977), up to 90 to 95% (Honey and McQuitty, 1979). Other studies in pig sheds have shown that concentrations of airborne particles vary over a 24 hour period, with levels peaking at 2.67 mg/m^3 around mid-morning, due to feeding and human activity, and decreasing to 0.42 mg/m^3 from midnight to dawn, when pigs are sleeping (Cargill *et al.,* 1997).

Heber *et al*., (1988) reported that non-respirable particles in pig buildings accounted for more than 80% in mass, but less than 30% in terms of count. A study by Cambra-Lopez (2011) reported that the mass (expressed as percentage of total dust) of thoracic dust (PM_{10}) was 30-54% and 99% in terms of count. For respirable dust ($PM_{2.5}$) the figures were 1-3% for mass and 90-99% in terms of count. The difference in the mass and numeric size distribution reflects the fact that small dust particles contribute little to mass.

High levels of dust can increase the severity of respiratory disease in pigs and may depress growth rates in the absence of respiratory disease (Cargill and Banhazi, 1996). Dust is not only an important respiratory irritant, but a carrier for other toxic pollutants found in pig sheds. Ammonia gas, micro-organisms and cell wall components from dead bacteria, such as endotoxins and β-1,3-glucan, are absorbed onto dust particles and carried into the respiratory tract. Dust from pig buildings can cause inflammation of the airways and is a cause of airways obstruction (Donham, 1995; Cargill and Banhazi, 1996).

2.5.1 *Source of particulate matter - dust*

Sources of dust in livestock production systems have been identified and assessed qualitatively and quantitatively (Donham and Gustafson, 1982; Aarnink *et al*., 1999). Dust particles within a livestock farming environment consist of up to 90% organic matter (Heber *et al*., 1988; Aarnink *et al*., 1999), which provides opportunities for bacteria and odouros components to adhere to these particles. Dust is likely to play the role as the carrier of the microorganisms in the air, because it appears that the microorganisms are associated with particle sizes larger than individual microorganisms (Zhao *et al*., 2009). The major source of dust is feed, but as most feed particles range from 10 to greater than 100 μm in diameter, feed has little effect on the concentration of respirable dust (Cargill and Skirrow, 1997). The contribution of feed to dust will depend on its composition and how it has been processed (Pearson and Sharples, 1995).

Sources of respirable particles tend to be dried faeces and urine, bedding, as well as skin dander from the pigs (Donham *et al*., 1986; Aarnink *et al*., 1999). Many respirable dust particles contain enteric bacteria and endotoxins, suggesting that they arise from faeces (Pickrell *et al.,* 1993). Included in respirable dust are a number of bioaerosols, such as undigested feed and gut epithelium, as well as feed components such as grain mites, antibiotics and growth promotants (Donham, 1995).

2.5.2 *Measuring particulate matter - dust*

The most common method for measuring respirable dust is to trap dust particles on filter paper inside a cassella cyclone attached to a vacuum pump operated for 3 to 8

hours at 1.9 l/min (Cargill and Skirrow, 1997). A similar method, which uses an open faced filter holder with protective cowl, called an IOM (Institute of Occupational Medicine), attached to a vacuum pump and operated at 2 l/min can be used to measure total dust. Filter papers are dried to remove moisture and weighed before and after use (Banhazi and Cargill, 1997).

Real-time particle counters, which are based on electro-optical methods and can be set to measure particles below a certain size (eg. PM10 for 10 μm or PM5 for 5 μm), can be used to monitor levels of dust over extended periods. This method is particularly useful in studying dust production in relation to staff and pig activities, such as weighing pigs, or in monitoring levels in 'before and after' studies when testing the efficacy of dust reducing treatments, such as misting with oil and water (Banhazi *et al*., 2008). These dust samplers can monitor the realtime dust concentrations, and no further process is needed after sampling (unlike the gravimetric method, in which filters must be weighed). Moreover, some optical samplers may record the concentrations of dust in different size ranges separately. However, the optical samplers have limitations in humid environments, because water droplets are also counted as dust particles. (Zhao *et al*., 2011). Due to ease of use and better reliability in environments with high dust loadings, particulate matter samplers that use a cyclone pre-separator are best for measuring particulate matter.

2.5.3 *Effects of particulate matter – dust, on pigs and humans*

The effects of dust on pigs and humans are difficult to quantify because of the nature and source of the dust. In most cases the dust will contain other bioaerosols, such as bacteria and endotoxins, as well as volatile fatty acids and gases such as ammonia.

There are three ways in which particulate matter might affect the health of pigs and humans: (1) by irritation of the respiratory tract and a consequent reduction of immune resistance to respiratory to respiratory diseases by particulate matter inhalation; (2) by irritation of the respiratory tract by certain compounds present in particulate matter; and (3) by inhalation of pathogenic and non-pathogenic microorganisms carried by particulate matter (Harry, 1978).

A number of studies have linked respirable dust levels in pig sheds to reduced growth rates and increased respiratory disease in pigs (Donham *et al*., 1989; Rylander *et al*., 1989; Skirrow *et al*., 1995). However, none of these studies have attempted to look at the effects of dust on pig health and production *per se*.

Numerous studies have demonstrated a link between dust and human health in a number of livestock related industries (Donham *et al*., 1989; Donham, 1995). A survey of 69 full-time poultry stockmen found that although levels of exposure to respirable dust were within occupational health and safety guidelines, 20% were exposed to levels of dust 2.5 times the figure of 10 mg/m^3 recommended under occupational health and safety guidelines (Whyte *et al*., 1994). Findings such as these have led to the

introduction of strict codes to protect people involved in the intensive livestock industries in several countries including Denmark, Sweden, The Netherlands, United States of America and Canada (Reynolds *et al*., 1996). Guidelines have also been recommended to the Australian pig industry (Jackson and Mahon, 1995).

The combined negative health effects of dust and ammonia in poultry housing are greater than their independent additive effects (Donham *et al*., 2002). Due to this synergistic effect it was concluded that the Occupational Safety and Health Administration (USA) exposure limits be reduced for ammonia (50 ppm to 7 ppm) and dust (15 mg/m^3 to 2.4 mg/m^3). The greater relative toxic nature of dust in poultry buildings is due to its high biological activity, its inflammatory nature, and the additive and synergistic actions of the mixed dust and ammonia exposures. It has been reported that nearly 60% of swine confinement workers who have worked for 6 or more years experience one or more respiratory symptoms (Clark *et al*., 1983; Donham *et al*., 1989). Prevalence of respiratory symptoms among workers in swine confinement buildings is 25%, compared with approximately 12% in non-confinement swine workers (Donham, 1990).

Studies in which bioaersols were measured as part of the dust component are described in Section 2.6.2.

2.5.4 *Controlling particulate matter - dust*

The strategies for reducing particulate matter inside pig buildings can be classified into two main groups: source-control techniques, which aim to reduce particulate matter emission from the source, and dilution and effective air room distribution (Amuhanna, 2007). These reduction strategies include using low-dust feed (Nannen *et al*., 2005), inclusion of feed additives (Guarino *et al*., 2007), water or oil spraying (Takai and Pedersen, 2000), changes in ventilation rate and air distribution (Gustafsson and von Wachenfelt, 2006) and vacuum cleaning (Nilsson, 1982). A number of studies have reported strategies to reduce particulate matter emissions from pig sheds into the atmosphere, including scrubbers, ionizers or electrostatic precipitators (Ogink and Aarnink, 2007; Ogink *et al*., 2008; Zhao *et al*., 2011a).

Automated enclosed feeding systems that deliver undamaged pelleted feed to the animals, have resulted in a reduction of the dust level. Unfortunately, in many automated feeding systems, pellets are damaged in transit, resulting in dust levels which are higher than in sheds feeding unpelleted diets (Cargill *et al.,* 1995; Banhazi *et al.*, 2000). Feeding systems that minimise the distance by which feed is dropped into an open container should help with reducing dust levels.

Other strategies for reducing dust within sheds include a reduction in the number or biomass of animals sharing the airspace, and regular cleaning of sheds by washing pens, floors, walls and ceilings with clean water (Banhazi *et al*., 2003). Industrial vacuum

cleaners have also been used in some countries to remove dust from pen walls and floors (Nilsson, 1982).

Fogging, showering and misting sheds with water have all been used to reduce dust particles. Although some positive effects have been observed in reducing total dust, there was only a minor reduction on respirable particles (Ryhr-Andersson, 1990).

Spraying pig sheds with water and oil mixtures has been shown to be effective in reducing dust and particulate matter (Nonnemann *et al*., 2004; Senthilselvan *et al*., 1997; Takei *et al.,* 1995; Gustafsson, 1994). The system requires an emollient, which can be operated from an existing sprinkler system with modified nozzles. The oil is added with an emollient into the water line via an automatic dosing system at a rate of 3 g oil/pig/day. Pigs and rooms are sprayed 5 to 10 times per day for 20 to 30 sec each time. Danish pig producers use canola oil, but oils other than canola oil have been used in Australia (Banhazi *et al*., 2002). A similar system, which sprinkles the shed with vegetable oil only, at a rate of 5 to 20 ml/m^2 of floorspace/day, has been developed in Canada (Zhang, 1996).

Adding soybean oil to the diet has been shown to reduce total dust levels, but has minimal effect on respirable dust (Mankell *et al*., 1995). Another study has shown that spraying feed with canola oil reduced total dust, but resulted in higher levels of respirable particles (Welford *et al*., 1992).

In a Canadian study (Senthilselvan *et al*., 1997), 20 human subjects naïve to pig shed environments were divided into two groups and exposed for 5 h/day over 2 days to either a shed sprinkled with canola oil, or left untreated. Several measurements, including Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), white blood cell counts, methacholine challenge and nasal cell counts, were obtained on a daily basis. Humans exposed to untreated buildings had a 10% shift change in FEV_1 which was associated with higher levels of ammonia, dust and endotoxin. The ammonia level in both environments was high; 26 ppm versus 18.3 ppm in the untreated and treated rooms, respectively. The dust level was 2.41 mg/m^3 in the untreated room and 0.15 mg/m^3 in the treated room and airborne endotoxin were 3,983 EU/m^3 in the untreated room and 452 EU/m^3 in the treated room.

Modifying ventilation systems, so that air inlets are at human head height and outlets are below slat level, has reduced the exposure level of humans to both respirable and total dust (Van't Klooster *et al.,* 1993). However, ventilation rates have a direct effect on dust levels through increased air movement, which increases re-suspension of particles and delays settling time. Animal movement also influence the airflow patterns in sheds and the fact that they form a solid mass in the path of the air (Smith *et al*., 1993). The key problem is that most ventilation systems are set for temperature control and, hence, too low to affect dust levels (Robertson, 1993). In another study it was demonstrated that ventilation only reduces airborne particle concentrations when the hygiene is good and the floors are cleaned. When hygiene is poor, increased ventilation actually increases the levels of airborne pollutants (Banhazi *et al.*, 2000).

Natural ventilation is based on the stack effect, ie. as warm air rises from the animals and the floor, it is exhausted through high level vents and fresh air is drawn in through low level openings. Hence capped ridge vents approximately 10% the width of the shed are helpful in maintaining adequate air exchange rates in naturally ventilated sheds (Clarke, 1994; Cargill *et al.,* 1999). However, while gas may be removed efficiently by natural ventilation, the capacity of such systems to remove dust is relatively poor unless a strong updraught is developed inside the building. There is a tendency for particles to be held in suspension without being removed (Cargill and Skirrow, 1997). Air filtration systems have been used, but these are difficult to assemble and operate in naturally ventilated sheds.

Ionisation of the airspace has been reported to increase the rate of dust deposition in sheds (Enache and Andrisan, 1990), but the method has not been widely examined under commercial production methods.

2.6 Particulate matter - airborne microbial load and bioaerosols

The airspace in any building housing animals will contain a collection of micro-organisms and their metabolites. These include both viable and non-viable bacteria, and fungal particles such as spores, sporangia and hyphae. A number of microbial cell wall components including endotoxins, β-1,3-glucan and peptidoglycan, as well as mycotoxins, microbial proteases, tannins and volatile fatty acids may also be present. Some of the micro-organisms will be free or in clumps and others, including the metabolites, toxins and volatile fatty acids, will be absorbed onto airborne particulate

matter. The latter are commonly referred to as bioaerosols (Donham, 1995; Cargill and Skirrow, 1997).

Most of the airborne microorganisms in livestock production systems are bacteria, of which the most dominant are gram-positive (Cormier *et al*., 1990; Skirrow *et al*., 1995). Airborne gram-positive Enterococci were found to account for up to 96% of the total bacteria recovered in poultry and pig houses (Clark *et al*., 1983). The most common species of these gram-positive bacteria are Staphylococcus, Streptococcus and Enterococci (Clark *et al*., 1983; Hartung, 1992; Matkovic *et al*., 2007). Gram-negative bacteria account for only a fraction of airborne bacteria (Zucker *et al*., 2000). Bakutis *et al*., (2004) reported that in terms of the total bacterial count, the proportion of gram-negative bacteria was 4.9% in pig houses. The proportion of fungi, moulds and yeasts in the airborne microbial flora in animal houses is low (Hartung, 1992).

While it has been reported that less than 10% of the organisms present are viable (Cargill and Skirrow, 1997), the cell wall components of some dead (or non-viable) bacteria are capable of engaging the immune system of pigs and humans (Donham, 1995). In an Australian study, large numbers of *Streptococcus* spp have been recovered in air samples from weaner, grower and finisher accommodation (Skirrow *et al*., 1995) and there is an association between the concentration of viable streptococcal organisms and the prevalence of pleurisy in pigs. It remains uncertain whether the level of streptococcal organisms is a direct cause of pleurisy or an indication of other factors that may predispose to it.

Recommended levels for viable airborne bacteria in pig sheds have been set at 100,000 colony forming units (cfu)/m^3 (Donham 1995; Pointon *et al*., 1995). However, it is difficult to quantify viable bacteria accurately as colony forming units are a measure of clumps of viable bacteria, rather than an individual bacterium.

Cormier *et al*., (1990) measured airborne micro-organisms in two types of swine confinement buildings (farrowing and fattening units) and recorded the predominant micro-organisms to be gram positive bacteria, with small quantities of gram negative bacteria, yeasts and moulds. Identification of the colonies revealed a great diversity of micro-organisms. Although there were some slight differences in airborne microbial flora in farrowing and fattening units, the level of airborne microbial contamination did not vary significantly as a function of the outside temperature. However, in another study (Butera *et al*., 1991), the temperature inside the room appeared to influence the concentration of viable bacteria, the latter being less at higher temperatures. There was a positive correlation between the humidity inside the room and respirable bioaerosols.

2.6.1 *Source of particulate matter - airborne microbial load and bioaerosols*

Pigs, their faeces, feed and bedding are the major sources of airborne microbial load and bioaerosols. The death of micro-organisms does not eliminate their cell wall components, such as endotoxins, β-1,3-glucan and peptidoglycan from the airspace (Wathes, 1994). It is generally accepted that all dust sources are also sources of airborne microorganisms because these source materials somehow contain certain microbial species that may be generated together with dust (Zhao *et al*., 2011).

2.6.2 Effects of particulate matter – airborne microbial load and bioaerosols on pigs

An important factor to consider when assessing the effects of pollutants on the health and production of pigs is that the different agents in particulate matter have different biological potency. The major health problem associated with intensively-housed pigs is an increase in the severity and prevalence of respiratory disease. A number of reports have linked higher levels of airborne bacteria with increased prevalence of respiratory lesions (Webster *et al*., 1987; Robertson *et al*., 1990; Donham, 1991; Hauser and Folsch, 1993; Clarke, 1994; Cargill and Skirrow, 1997; Madec, 2005). In studies completed in Australia (Cargill *et al.*, 1998), the severity of pneumonia in pigs examined at slaughter was reduced by 36% and pleurisy prevalence by 25% in pigs reared in sheds with an acceptable level of bacteria compared with pigs reared in sheds with unacceptable bacteria levels. There was also a strong correlation between concentrations of airborne gram positive bacteria and both the prevalence of pleurisy and severity of pneumonia (Skirrow *et al*., 1995). Airborne particles may also act as vectors in the spread of other infections between buildings housing livestock, and it has been suggested that buildings need to be at least 100 to 150 metres apart to prevent dust-driven disease spread (Collins and Algers, 1986). This distance is supported by Duan *et al*., (2009), who could not detect *E. coli* strains in air samples taken 200 metres from pig buildings.

The key effects of poor air quality, identified with high levels of bacteria, include a range of clinical signs and inflammatory and immune responses. Clinical signs include

coughing, sneezing, salivation, loss of appetite and excessive lachrymal secretions, as well as depression of growth rate, which is not limited to pigs with respiratory disease (Donham, 1991; Skirrow *et al*., 1995). The inflammatory response is both local and general and involves activation of the immune system. Local inflammatory changes include loss of cilia, thickened epithelia and decreased numbers of goblet cells in the trachea and turbinates, along with activation of epithelial cells, alveolar macrophages, and polymorphonuclear cells releasing a variety of inflammatory mediators (Robinson, 1994). Non-specific activation of the immune system involves the production of cytokines and is thought to divert nutrients away from growth and accretion of skeletal muscle to support the inflammatory and immune responses (Almond *et al*., 1996; Johnson, 1998; Black *et al*., 2001; Escobar *et al*., 2004). The influence of immune activation on growth has been well documented in the pig and poultry industries (Kelley *et al*., 1987; Klasing and Barnes, 1988; Gotz *et al*., 2001; Le Floc'h *et al*., 2004) resulting in reduced weight gain, increased muscle protein degradation, decreased protein synthesis, and reduced muscle protein accretion.

In a study by Crowe *et al.,* (1994), it was found that pigs reared in isolated all-in/all-out (AIAO) nurseries (*Isowean*) were heavier at the end of the study than littermates weaned in conventional farm nurseries. The *Isowean* environments also had less dust and endotoxin levels than the conventional environments. It was suggested that these low levels of pollutants were achieved by rigorous cleaning and disinfecting of the facilities between batches (Crowe *et al*., 1994) and that a possible explanation for the improvement in growth involved decreased stimulation of the immune system. The

importance of cleaning has also been demonstrated in other studies (Knowles *et al*., 1997; Cargill and Banhazi, 1998; Lee *et al*., 2005; Le Floc'h *et al*., 2006) where pigs reared in clean rooms grew 8 to 10% faster than pigs reared in uncleaned, or dirty rooms. The clean shed was disinfected thoroughly prior to stocking and maintained in a clean state by daily hosing of pens and aisles, flushing effluent channels with clean water, and twice daily fogging of the air space with a virucidal agent (Virkon S®, Antec International, Suffolk, UK). The dirty environment was achieved by not cleaning the shed prior to stocking, or throughout the experiment and by recycling effluent continuously beneath the floor slats. Increased phagocytic activity and impaired proliferative lymphocyte responses were also demonstrated in pigs reared in the dirty environments (Lee *et al*., 2005).

Harris *et al*., (1990) observed that pigs raised in the *Isowean* system had larger thymus glands (as a percentage of body weight) than conventionally reared littermates. These pigs also appeared to have smaller peripheral lymph nodes compared to controls and a greater proportion of CD4/CD8 lymphocytes in the circulating blood. Lymphocyte migration was also decreased in *Isowean* pigs. This could imply that pigs raised in the cleaner *'Isowean'* system did not have their immune system stimulated to the extent of litter mates raised in conventional environments. Besides pathogen stimulation of the pig's immune system, other antigens, such as endotoxin and antigens found in dust, could be responsible for the immune stimulation observed in conventionally reared pigs.

2.6.3 Effects of particulate matter – airborne microbial load and bioaerosols on humans

The pig industry has been identified as having risk factors which cause injury to workers (Rautiainen *et al*., 2009). Numerous studies have identified risk factors for airway diseases in pig farmers (Schwartz *et al*., 1995; Von Essen *et al*., 1998; Radon *et al*., 2001; Radon *et al*., 2002; Radon *et al*., 2002a; Andersen *et al*., 2004) and demonstrated links between dust in pig sheds and human health (Donham, 1990; Gerber *et al*., 1991; Hartung and Phillips, 1994; Donham, 2000; Cargill and Hartung, 2001; Radon *et al*., 2002a).

Many of the studies presented below did not analyse the particulate matter the pig workers were exposed to. Other studies did analyse gases and particulate matter, however, it is difficult to extrapolate which component of the airborne pollutants, had the greatest effect on worker health.

The first report indicating health hazards for humans working in intensive livestock production systems was published over 30 years ago (Donham *et al*., 1977). Since then a number of studies from various countries including Canada (Holness *et al*., 1987; Dosman *et al*., 1988), Sweden (Donham, 1986; Donham *et al*., 1986; Hagland and Rylander, 1987), The Netherlands (Brouwer *et al*., 1986; Bongers *et al*., 1987) and the United States of America (Donham *et al*., 1982; Donham *et al*, 1984; Donham *et al*., 1986) have investigated the acute and chronic respiratory function in workers employed in the intensive livestock industry. Although some workers may have adverse

respiratory symptoms within the first week of work, most will not develop symptoms unless they have worked in intensive production systems for more than 2 hours per day and for 6 or more years (Donham and Gustafson, 1982).

The results of several surveys (Dosman *et al*., 1988; Donham, 1990; Cargill and Hartung, 2001; Radon *et al*., 2002) indicate that acute bronchitis is the most common syndrome in the pig industry followed by Organic Dust Toxic Syndrome (ODTS) and Occupational Asthma (Table 2.5). Bronchitis is also the most common complaint registered and symptoms usually occur in workers exposed to dust for longer than two hours each day. In another study, the prevalence of asthma-like symptoms in the pig industry was 39%, compared with 5% in the dairy industry (Iversen and Pedersen, 1990).

The risk of acute and chronic respiratory health effects of those working in the intensive livestock industry is apparently determined by a number of factors. Some of these include the concentration of airborne pollutants in the airspace, pre-existing respiratory conditions, length of time the person has worked and their susceptibility to endotoxin or allergens in the airspace (Donham, 2010).

Table 2.5: Results of studies completed in Australia, Finland, Denmark, Sweden, Scotland, and North America showing percentage of workers in the intensive livestock industries with occupational respiratory problems (Choiniere and Munroe, 1994).

Acute symptoms (including cough, phlegm, scratchy throat, runny nose, burning or watering eyes, headaches, tightness of chest, shortness of breath, wheezing and muscle aches) were studied in pig industry workers from Denmark, Sweden, The Netherlands and the United States of America (Donham *et al*., 1977; Brouwer *et al*., 1986; Iverson *et al*., 1988; Donham *et al*. 1989). Acute symptoms were defined as those which the worker directly associated with the working environment. The symptoms of those working in the intensive pig industry were at least twice as prevalent as the non-farming

controls, and almost 50% higher than pig producers not using confinement facilities. The prevalence of the acute symptoms was 20-50% for wheeze and tightness of chest and 18-75% for cough. The non-farming control populations used in these studies were farmers not raising pigs, or raising pigs in non-confinement facilities or workers considered to be in a 'clean air' environment, such as postal workers (Brouwer *et al*., 1986; Donham *et al*., 1989).

The prevalence of chronic symptoms (cough, phlegm, wheeze, tightness of chest, and shortness of breath) in a number of studies (Donham *et al*., 1982; Donham *et al*, 1984; Donham, 1986; Brouwer *et al*., 1986; Bongers *et al*., 1987; Haglind and Rylander, 1987; Holness *et al*., 1987; Dosman *et al*., 1988) was from two to four times greater than that found in the control populations. The prevalence of cough ranged from to 20-50%, phlegm ranged from 12-55% and wheeze, tightness of chest and shortness of breath ranged from 12-33%.

A range of symptoms, including cough, headaches, malaise, nausea, nasal stuffiness and moderate chills were experienced by healthy, previously unexposed volunteers to several hours of swine dust in pig sheds (Cormier *et al*., 1997; Larsson *et al*., 1997; Muller-Suur *et al*., 1997). Similar symptoms, including eye irritation, tiredness, throat irritation and flulike symptoms were experienced by third-year veterinary students exposed to swine dust in pig sheds for 3 hours (Jolie *et al*., 1998). These symptoms developed on the same day of exposure and disappeared within 3 days. Healthy

subjects exposed to bacterial endotoxin reported symptoms of cough, headache, throat irritation and lethargy 24 hours after exposure (Thorn and Rylander, 1998).

A study by Donham *et al*., (1995) has reported that the relationship between exposure and decline in pulmonary function in 207 swine confinement farmers was highest after 6 years of exposure to airborne pollutants; the strongest predictors being total dust and ammonia. This cohort of swine confinement farmers was followed-up in a subsequent study 48 months later by Reynolds *et al*., (1996) who found the strongest correlations in workers who had 0-6 years or 10-13 years of pollutant exposure. Total and respirable endotoxins and ammonia were strongly correlated with a decline in pulmonary function in the 0- to 6-year group, while total dust, respirable dust and ammonia correlated with a decline in pulmonary function in the 10- to 13-year group. These results suggest that while dust may be an important factor for chronic changes in pulmonary function, endotoxin may be the most important for acute effects.

Studies of baseline pulmonary function suggest a small, but non-significant, average decrease in pulmonary values, as well as forced expiratory volume-in-one-second (FEV_1)/forced vital capacity (FVC) in swine confinement workers compared to standard non-farming urban control populations (Donham *et al*., 1984; Bongers *et al*., 1987; Haglind and Rylander, 1987; Dosman *et al*., 1988). A study by Schwartz *et al*., (1990) found that flow rates at 25 to 75% of lung volume (FEF_{25-75}) were significantly less than the control population. Futhermore, work-shift (period of time a person is working on a

particular day) reductions in FEV_1 and flow rate values are seen in most confinement house workers after a 2- to 4-hour exposure.

Exposure to bioaerosols has also been shown to cause broncho-constriction, airway hyper-responsiveness and increased inflammatory cells in bronchial alveolar lavage fluids in naïve subjects (Malmberg and Larsson, 1993). Post-exposure levels of blood leucocytes and neutrophil granulocytes in broncheoalveolar lavage (BAL) were 75 times greater than pre-exposure levels, and orosomucoid and C-reactive protein were significantly elevated after one day. Increased amounts of lymphokines were also found in the lower airways, as a result of activated lymphocytes. While one three hour exposure of naïve patients to 'pig dust' increased bronchial reactivity for more than one week, the FEV_1 did not change significantly. These findings may help explain why workers complain of general malaise without reporting loss of lung function. However, the broncho-constrictive effects of bioaerosols have also been demonstrated in guinea pigs (Zuskin *et al.,* 1991) as well as stockpersons in Sweden and North America (Donham, 1995).

Although several studies have demonstrated increased levels of both Immunoglobulin (Ig) G and IgE antibodies to common environmental allergens, no differences were found between populations of piggery staff and control populations. Correlations between IgG levels and clinical signs could not be demonstrated. However two studies found an association between IgE and other factors. In one study there was an association between IgE to environmental allergens and the number of hours worked in

a shed (Brouwer *et al.,* 1986), and in the other there was an association between IgE to dust and increased broncho-constriction in three workers (Zuskin *et al.,* 1991). No associations between skin-prick tests and clinical disease have been demonstrated (Donham, 1995). A study conducted amongst 122 pig veterinarians showed a number of work-related symptoms including rhinitis, cough and chest tightness, wheezing and airway obstruction (Andersen *et al*., 2004).

There are potential limitations in the human studies mentioned in 2.6.3, which link exposures to a single, or multiple, pollutant exposures to symptoms and lung function indices. Often, the pollutant measurements are obtained on a single day and used to compare with symptoms or lung function tests. It is known that pollutant concentrations vary spatially and by shift, day, week and season (Subramanian *et al*., 1996; Donham *et al*., 2002; Banhazi *et al*., 2004). Therefore, isolated short-term pollutant measurements are being compared with health effects that may result from long-term exposures. The short-term measurements may not be representative of the actual exposures to workers over time. The studies are not consistent whether they obtained pollutant measurements from personal samplers or from room samples. The latter may be poor estimators of personal exposure. It is also known that pollutant concentration vary between sheds on the same farm (Backstrom and Jolie, 1996).

2.6.4 *Measuring particulate matter - airborne microbial load and bioaerosols*

The methods used to measure bioaerosols in pig sheds have been reviewed by Thorne *et al.*, (1992). Samplers are generally based on one of three main principles, ie. impaction,

impingement, or filtration. An example of the impaction principle is the Andersen sampler (Andersen Instruments Incorporated, Atlanta, USA) which can be used to distinguish microorganisms according to their size; it does not count the microorganisms (Andersen, 1958). The Andersen sampler, attached to a vacuum pump, is designed to trap bacterial particles onto plates containing solid culture media. The sampler has 6 stages or levels, with the heaviest and largest particles being deposited onto the first stage and the smallest onto the last (sixth) stage. The plates are then incubated and the number of bacteria estimated as colony forming units (cfu's)/m^3 airspace. The Andersen impactor may easily become overloaded when samples are taken in livestock houses (Thorne *et al*., 1992), therefore, the sampling duration is limited to minutes or even seconds. An example of the impingement principle is the AGI-30 (Ace Glass, Vineland, USA). Particulate matter is collected using liquid impingers followed by dilution plating onto a variety of media to quantify various microbial groups. A disadvantage of this sampler is that it may not be able to sample for a long time due to evaporation of the collection liquid (Lin *et al*., 1997). The recommended maximum duration of the sampling period for the AGI-30 is 30 minutes (Zhao *et al*., 2009). Total micro-organism counts can be measured using a nucleopore filtration-elution method. An example of the filtration method is a dissolvable gelatin filter (Sartorius, Gottingen, Germany). Filters are used to trap dust and other bioaerosols, washed, and the fluid examined for cells. This method provides an estimate of the total number of bacterial cells present in the environment, rather than just viable organisms as measured with the Andersen Sampler (Thorne *et al.*, 1992). The filtration method is not suitable for sampling microorganisms that are vulnerable to

dehydration stress (Zhao *et al*., 2009). Techniques using the filters to collect respirable dust, are also available for estimating the concentration of endotoxins, β-1,3-glucan and peptidoglycan (Backstrom and Jolie, 1996). For organisms that cannot be cultured, a number of methods have been developed, including direct count with DNA staining and epifluorescence microscopy, fluorescent in situ hybridisation and PCR techniques (Thorne *et al*., 1992).

2.6.5 *Reducing particulate matter - airborne microbial load and bioaerosols*

As with particulate matter/dust, there are a number of ways to reduce microbial load and bioaerosols from pig shed. These include source-control techniques, which aim at reducing the pollutants from the source, and improving building factors such as effluent removal and ventilation.

Improving hygiene in sheds by improving effluent disposal systems, correcting dunging patterns, and cleaning pens and pits, results in lower levels of both airborne respirable particles and bacteria. Converting existing continuous-flow (CF) production systems to all-in/all-out (AIAO) management will also improve hygiene standards in pig buildings (Cargill *et al.*, 1997).

Studies have shown that bioaerosol emissions can be reduced by installing biofilters or bioscrubbers (Zhao *et al*., 2011a). These units have been developed to reduce ammonia emissions and bioaerosol emissions. These units are associated with high-energy costs and frequent maintenance to guarantee cleaning efficiency.

2.7 Endotoxin, β-1,3 glucan and peptidoglycan

Endotoxin (lipopolysaccharide; LPS) is a constituent of the outer membrane of Gram-negative bacteria and an important microbial trigger that stimulates innate immunity (Fearon and Locksley, 1996; Medzhitov and Janeway, 1997). The individual immune response to endotoxin is the result of a complex interaction between dose and timing of exposure, additive or synergistic effects, and genetic predisposition (Vandenbulcke *et al*., 2005). β-1,3-glucan and peptidoglycan are cell wall components of gram positive bacteria and fungi, and all bacteria, respectively (Donham, 1995).

Studies which differentiated inhalable and respirable dust fractions, described that endotoxins were found in both fractions but with predominance in the inhalable fraction (Mandryk *et al*., 1999; Nieuwenhuijsen *et al*., 1999; Donham *et al*., 2000).

A number of studies from the Netherlands (Castellan *et al*., 1984; Castellan *et al*., 1987) have proposed a health-based recommended threshold value for endotoxin of 50 EU/m^3 (endotoxin units) which equals 5 ng/m^3. This value was derived from a single endotoxin exposure. In another study (Backstrom and Jolie, 1996), recommended levels for endotoxins in pig sheds to 0.08 ng/m^3 for humans and 0.15 ng/m^3 for pigs. No recommended levels have been set for β-1,3-glucan and peptidoglycan.

A study by Backstrom and Jolie (1996) observed that peptidoglycan levels in weaner pig rooms were double those in other areas of the farm, while endotoxin levels were three times higher in dry sow housing than in other areas. This suggests that different

pollutants may contribute to poor air quality in different areas of the farm to different classes of pigs at different stages of life.

Studies which have quantified concentrations of endotoxins recorded in livestock units in Europe (Carpenter, 1986) and Australia (Currie *et al*., 1997) indicate that levels in livestock production sheds are often above maximum recommended values and that they are generally higher in poultry units than in other forms of livestock production. Values of 45.1 ng/m^3 have been found in the inhalable dust fraction and 7.5 ng/m^3 in the respirable fraction (Seedorf *et al*., 1998). Levels in pig sheds and cattle barns were 10.5 and 6.1 and 9.6 and 0.4 ng/m^3 respectively. Studies in Australian pig and poultry sheds suggest that similar levels are common (Cargill and Hartung, 2001), but in other studies much higher levels were recorded (Currie *et al.*, 1997). Endotoxin levels recorded in a study involving 153 pig sheds ranged from zero to 23.8 ng/m^3 with a mean of 3.3 ng/m^3 (Banhazi *et al.*, 2000). The highest levels were recorded in straw-based shelters (8.5 ng/m^3) with levels in other classes of housing ranging from 2.3 ng/m3 in dry sow sheds to 3.4 ng/m^3 in finisher sheds. The key factors associated with higher levels of endotoxins were reported to be continuous flow production (as opposed to all-in/all-out production with cleaning), straw based shelters and mechanical ventilation (Banhazi *et al*., 2000).

2.7.1 Effects of endotoxins on pigs

Aerosol exposure to endotoxin and/or β-1,3-glucan influences the cell population present in the respiratory tract and influences lysosomal enzyme production by

macrophages (Fogelmark *et al.*, 1994). It is hypothesised that chronic exposure of pigs to dust, endotoxin, and β-1,3-glucan induces inflammatory changes in the respiratory tract leading to impaired disease resistance. The immunological challenge impairs metabolism intended for growth and skeletal muscle accretion in order to enhance metabolic processes that support the immune response (Le Floc'h *et al*., 2004). The alteration in metabolism involves a decrease in Insulin Growth Factor-1 (IGF-1) concentrations. It is for this reason that dietary manipulation generally fails to improve pig growth after immunological challenge (Black *et al*., 2001).

Several in vivo and in vitro studies in guinea pigs have demonstrated that endotoxins, moulds, and organic dust activate epithelial cells and alveolar macrophages (Rylander and Beijer, 1987). Aerosol exposure to endotoxins and β-1,3-glucan also modifies the cell population present in the respiratory tract (Fogelmark *et al*., 1994).

2.7.2 Effect of endotoxin on humans

There is considerable variation in the reported response to inhaled LPS in the literature. This variation could be attributable to a number of factors, including the type of the endotoxin used (*E. coli* or *Enterobacter agglomerans*), age, gender, smoking, and the individual's expression of CD14 and LPS-binding protein (Alexis *et al*., 2001).

Health effects of endotoxin exposure can be described as paradoxical (Liu, 2002; Radon, 2006); positive, as well as negative, health effects have been described in humans. Negative effects with endotoxin exposure have been described with symptoms

including fever, cough, shortness of breath, wheezing, headache, nose and throat irritation, chest tightness, acute airway flow restriction, and inflammation (Burrell and Ye, 1990; Heederik *et al*., 1991; Douwes and Heederik, 1997). Endotoxin can increase disease severity by acting as a natural adjuvant to augment asthma and atopic inflammation, or may act on its own, causing adverse effects on lung function and inflammatory responses (Liebers *et al*., 2006). Positive effects have been described especially with respect to development of allergies (von Mutius *et al*, 2000; Braun-Fahrlander 2002; Eduard *et al*., 2004). Less well documented are positive effects with regard to cancer risk (Lange, 2000; Mastrangelo *et al*., 2005).

In a 3-year follow-up study by Vogelzang *et al*., (1998), pig farmers recorded a reduction in FEV_1 and FVC which was associated with endotoxin exposure. Total dust exposure was associated with decreased FVC only. These results did not differ between symptomatic and asymptomatic workers.

The field studies described above have not differentiated between effects of single components. However, FEV_1 decrease and inflammatory response due to endotoxin inhalation has been shown in clinical challenge experiments (Kitz *et al*., 2006) verifying that endotoxin is a potential inducer of adverse health effects. However, a study by Kitz *et al*., (2008) reported only minor clinical reactions to endotoxin exposure including cough, headache, chills and fatigue. These adverse events resolved spontaneously within 10 hours. There were no significant differences in FEV_1.

The dose of endotoxin responsible for immunological mediators leading to T_H1 or T_H2 responses is poorly characterised. A study using an animal model suggested that doses of LPS as low as 0.1 mg lead to TH2 type responses (atopic), which involves the release of eosinophils, IL-5 and IL-13 (Eisenbarth *et al*., 2002) and an increase in phagocyte function (Alexis *et al*., 2004).

Although measurement of endotoxins is hitherto incompletely standardised (Liebers *et al*., 2006; Spaan *et al*., 2007), it is clear that endotoxin exposure can cause acute and chronic health effects (Rylander, 2006).

2.8 Immune system of the pig

The porcine immune system is comprised of many components that respond in a coordinated way to defend the animal against infections. Resistance to pathogens is provided by both innate or natural immunity and specific or acquired immunity (Corbeil, 1991; Roth, 1992).

The immune responses of the upper and lower respiratory tracts differ in their defence mechanisms. The upper tract can be best described as the first line of defence, filtering out potentially hazardous substances before they reach the lower respiratory tract (Taylor, 1996).

The upper respiratory tract, predominantly the nasal cavity, warms, humidifies and filters the air. This is aided by the presence of dorsal and ventral turbinates which

greatly increase the surface area of the nasal cavity mucosa (Taylor, 1996). As the pig inhales, air is forced through the turbinates in a circular motion forcing any large particles inhaled into the nostril hairs where they are trapped in mucus (Christensen and Mousing, 1992). Particles greater than 5 μm in diameter are generally trapped by the epithelial mucus of either the nasal, pharyngeal, laryngeal or tracheal cavities before they pass beyond the tracheal bifurcation (Taylor, 1996; Christensen and Mousing, 1992; Wilkie, 1982). Particles deposited on the epithelial mucous are eliminated by the mucociliary clearance mechanism, which delivers mucous to the pharyngeal cavity where it is swallowed.

The lung is the internal body organ with the most extensive environmental exposure and the most intimate contact between tissue, blood and the atmosphere (Jericho, 1968). Yet, despite continuous exposure, the normal bronchopulmonary system is able to maintain its sterility. The lung of the pig clears more bacteria from the blood than the liver or spleen due to a huge number of pulmonary intravascular macrophages which cover 16% of the lung capillary surface (Pabst and Binns, 1994). The basic defense mechanism of the lung relies on clearance of particles within the bronchial tree by the muco-ciliary apparatus, and phagocytosis of those particles that deposit in the alveoli by the alveolar macrophages. The lung contains large numbers of lymphocytes found in different compartments: (1) the pulmonary intravascular pool, which is organ-specific and shows a unique migration pattern; (2) the interstitial lymphocyte pool, which is equivalent to the whole blood pool; (3) the bronchus-associated lymphoid tissue (BALT) which develops as a result of microbial stimulation; (4) the intraepithelial and

lamina propria lymphocytes of the bronchi, with their typical subset composition; and (5) the lymphocytes in the bronchoalveolar space, which can be sampled by bronchoalveolar lavage (Pabst and Binns, 1994). The major immunoglobulin class in the lung is IgG. In conditions of optimal air quality, the respiratory system of pigs is able to eliminate 99% of a given exposure of *Staphyloccus aureus* within 6 hours, and 99.9% of a given exposure of *Pasteurella multocida* within 24 hours. This clearance is partly related to the rate of decay of the bacteria after aerosolization (Baekbo, 1998). Bioaerosols (particularly dust) and gases (ammonia) have an impact on the ability of these two systems to function optimally.

2.9 Research leading up to this project

In 1990, data obtained from the South Australian and Western Australian Pig Health Monitoring Schemes confirmed that the prevalence of respiratory disease in pigs, especially pleurisy, was relatively high and increasing annually (Skirrow *et al*., 1995). Pleurisy, or inflammation of the pleura, is usually caused by bacterial infection, particularly *Actinobacillus pleuropneumoniae,* and leads to adhesions either between adjacent lung lobes or in more severe cases, between the thoracic wall and the lung (Pointon *et al*., 1995). As the condition produces obvious lesions, it is easily detected during slaughter inspection (Pointon *et al*., 1995).

Subsequently, the results of an objective study into the causes and risk factors associated with pleurisy in pigs on Australian farms demonstrated that although a range of pathogens were involved, the prevalence of pleurisy in a herd was associated directly

with a number of husbandry and environmental factors (Skirrow *et al*., 1995). The most significant factors associated with increased pleurisy prevalence were the concentration of airborne streptococcal organisms in the pig shed and the concentration of airborne respirable dust. Other significant factors included the stocking density (m^3 airspace/pig) and the number of pigs sharing the same airspace (Skirrow *et al*., 1995). It was also found that stocking density levels were below the recommended level on a majority of farms. The authors also reported that the number of pigs in an airspace (shed population) was not only positively correlated with the prevalence of pleurisy and pneumonia in slaughter pigs, as well as coughing rates in pigs on the farm, but also with the concentration of airborne respirable dust and the bacterial load in the airspace (Skirrow *et al*., 1995). On the other hand, the stocking density (m^3 airspace/pig) was not only negatively correlated with the prevalence of pleurisy, but the bacterial load and the concentration of airborne streptococcal organisms in the airspace as well.

In other studies completed in Australia, the severity of pneumonia in pigs examined at slaughter was reduced by 36% and pleurisy prevalence by 25% in pigs reared in sheds with acceptable air quality, compared with pigs reared in sheds with poor air quality (Cargill *et al*., 1998). There was also a significant association between concentrations of airborne gram-positive bacteria and the prevalence of pleurisy and severity of pneumonia.

3

The effects of ammonia and alpha haemolytic cocci (AHC) on feed intake, immune function and physiology in pigs

3.1 Introduction

There is strong evidence that many of the factors (social, climatic and hygienic) which reduce the performance of pigs raised in commercial environments, increase the stress level of the pigs. If this is the case, the removal of one, or more, stressor should have a positive effect on performance (Black *et al*., 2001).

The stresses arising from poor hygiene and poor air quality in intensive animal housing represent major concerns to producers, employees, housing and farming specialists, and veterinarians involved in the intensive livestock farming industries. A number of reports have highlighted the negative effects of sub-optimal air quality and hygiene on the health and production of animals, as well as the health of workers (Iversen and Pedersen, 1990; Hartung and Phillips, 1994; Donham, 1995; Knowles *et al*., 1997; Cargill *et al*., 1999; Murphy *et al*., 2000; Cargill and Hartung 2001; Radon *et al*., 2002; Le Floc'h *et al*., 2009).

Added to these concerns, the rate of growth, and the efficiency of feed eaten by pigs raised under commercial conditions in Australia are well below their genetic potential and the values that could be achieved if the animals were housed under 'ideal' conditions (Black and Carr, 1993). This difference has a significant impact on the profitability of a pig enterprise. There are many factors within a commercial piggery environment that may contribute to the reduction in feed intake, growth rate and efficiency of feed use and can act to increase the stress level of the pigs.

Air pollutants within pig buildings include microorganisms, their endotoxic cell-wall fragments, and ammonia (Banhazi *et al*., 2008). Interactions between these pollutants may arise because of a physical relationship, such as the adsorption of ammonia onto dust particles (Kim *et al*., 2005), or because of a pathological synergy (Drummond *et al*., 1978; Gustin *et al*., 1994; Johannsen *et al*., 1987; Urbain *et al*., 1996b).

The link between poor air quality and poor growth rates may be mediated through an effect of pollutants on the animals' immune function. Many studies (Knowles *et al*., 1997; Le Floc'h *et al*., 2009) have shown that poor sanitary conditions in pig sheds are associated with the induction of inflammatory responses, and that the inflammatory activation leads to slower growth. In part, the slower growth arises from a reduced voluntary food intake (VFI) (Escobar *et al*., 2004; Renaudeau, 2009) and suppression in food conversion efficiency (Le Floc'h *et al*., 2009). Both Le Floc'h *et al*., (2004) and Sandberg *et al*., (2007) concluded that the immune response *per se* is associated with a nutrient demand. The mediators, proinflammatory cytokines such as interleukin (IL)-1β released by activated mononuclear immunocytes (Johnson, 2002; Le Floc'h *et al*., 2004), have also been shown to initiate catabolism of skeletal muscle (Dionissopoulos *et al*., 2006). Indicators of such a cellular immune response are acid glycoproteins (Greiner *et al*., 2000; Grellner *et al*., 2002; Sauber *et al*., 1999) and the CD4:CD8 ratio of T lymphocytes (Davis *et al*., 2004).

Ammonia is highly water soluble and reacts with the moisture on mucosal surfaces to form a corrosive alkaline solution of ammonium hydroxide that irritates these surfaces

(Brautbar, 1998) causing epithelial hyperplasia and loss of cilia after prolonged exposure (Urbain *et al*., 1996a). Its solubility, however, means that most of the gas is absorbed in the nasopharynx, and little penetrates the lungs (Urbain *et al*., 1996a). It is the most important gaseous pollutant in pig sheds (Hartung, 1998; Subramanian *et al*., 1996), and at levels which occur commonly in pig sheds has been shown to cause damage to respiratory epithelia (Hamilton *et al*., 1998a; Urbain *et al*., 1996a), to induce inflammatory responses in the respiratory system (von Borell *et al*., 2007), and to suppress the cough reflex (Moreaux *et al*., 2000). An effect of even short-term exposure is to depress the defence mechanisms of the respiratory tract against inhaled microorganisms (Gustin *et al*., 1991) such as Pasteurella multocida (Hamilton *et al*., 1999).

As outlined in Chapter 2, air pollutants and sub-standard air quality have been identified as major factors in reducing growth rate efficiency in commercial production units. The key pollutants identified in previous studies were ammonia and airborne bacteria, especially *Streptococcus* sp, commonly referred to as 'faecal streps' because they are found in faeces (Cargill and Skirrow, 1997).

The purpose of this study was to examine the impact of the interactions between acute simultaneous exposure to ammonia gas and to a respiratory tract commensal on parameters of growth rate and feed conversion ratio (FCR), and on immune system parameters. I was aware that Andreason *et al*., (2000) had detected no synergistic effect between ammonia and a mixed inoculum of toxigenic *Mycoplasma hyopneumoniae* and

Pasteurella multocida, and I did not want to use potent pathogens that might obscure the impact of ammonia. I chose alpha haemolytic cocci (AHC) including viridans-group streptococci (VGS) as the model organisms because they have been isolated from gut samples of faecal slurries of some species (VGS, Thanantong *et al*., 2006; *Aerococcus viridans*, Budzinska *et al*., 2009, Byrne-Bailey 2009, Guo *et al*., 2007), because they are prevalent in the airspace of pig sheds (Done *et al*., 2005) including Australian sheds (Cargill and Skirrow, 1997), because they are considered to be generally non-pathogenic (VGS, Van der Hoeven and Camp, 1991; *Aerococcus viridans*, Park *et al*., 2005), and because they have been found, in some host species, to be upper respiratory-tract commensals (VGS, Van der Hoeven and Camp, 1991; *Aerococcus viridans*, Silvanose *et al*., 2001). In humans, VGS usually act as commensals utilising mucin as an energy substrate (Van der Hoeven and Camp, 1991), but may act as periodontal pathogens (Robertson and Smith, 2009) and may cause rhinosinusitis (Hwang and Tan, 2007). They are also common secondary colonisers in the distal airways of people with chronic lung diseases (Cabello *et al*., 1997). The factors that make the organisms pathogenic are not known (Hwang and Tan, 2007), but it is known that VGS represent a particular risk to humans with neutropaenia (Rieske *et al*., 1997; Tunkel and Sepkowitz, 2002), and that in pigs it readily colonises the aortic valve following mechanical damage to the valve, resulting in endocarditis (Johnson and Bowie, 1992; Ramirez-Ronda, 1978). Hence, I deemed them as suitable candidate organisms for a trial exploring the interactions between ammonia and bacteria. AHC are known to produce endotoxins (Hanage and Cohen, 2002), but I chose to use viable

organisms because they retain their potential to increase in numbers exponentially and to colonise new tissues if the environment becomes favourable.

3.2 Material and methods

3.2.1 *Research site*

The experiment was conducted in Research Room 4 of the University of Adelaide, Roseworthy Campus Research Piggery (Farm 1). The room was 12 m long, 8 m wide and 4 m high, a total volume of 384 m^3. An air conditioner system provided fresh, filtered air into the room under positive pressure and 2 computer-controlled exhaust fans expelled air from the room into the atmosphere. The temperature and humidity of the room were computer controlled and set at 24 ^{O}C and 55% relative humidity. The room consisted of 20 individual pens (2 rows of 10), each 1.6 m^2 with a partially slatted floor. Each pen had a covered feed bin (to prevent contamination by faeces and urine), which could be removed to collect orts. The room was cleaned and disinfected thoroughly between each trial. The room was cleaned three times a day with fresh potable water via a hose to remove urine and faecal material into the pit below the pen slats. The pit was flushed every second day, while the pigs were out of the room. Pigs with any visible faecal matter on their skin were washed and dried before being returned to their pen. Other stressors, such as stocking rate, stocking density, background ammonia and bacterial levels were minimised.

3.2.2 *Experimental animals*

Female (Large White x Landrace) respiratory disease-free gilts (16 weeks of age) were sourced from the Pig and Poultry Production Institute's (PPPI) herd housed in the University of Adelaide, Roseworthy Campus Research Piggery (Farm 1). Australia is free from transmissible gastroenteritis and porcine reproductive and respiratory syndrome. This piggery is free of helminth parasites, swine dysentery, and atrophic rhinitis. Mycoplasmal pneumonia, erysipelas, Glasser's Disease, leptospirosis, and clostridial diseases are controlled through a program of vaccinations.

Pigs were weighed daily, given access to water at all times and fed a daily ration of 3.0 kg of a commercial finisher diet (Lienert Australia, Roseworthy) consisting 13% protein, 2% fat, 6% fibre, 13MJ/kg digestible energy, divided into equal portions (morning and afternoon). Orts were collected and weighed. Voluntary feed intake (VFI), average daily weight gain (ADG), and food conversion ratio (FCR) were calculated. The daily feed loss due to immune challenge was calculated as the product of the mean VFI of the challenged animals minus the product of the mean FCR of the unchallenged animals and the mean ADG of the challenged animals.

3.2.3 *Experimental design*

The study was conducted as a 2 x 4 factorial with the main effects of bacterial challenge (control or alpha haemolytic cocci (AHC)) and ammonia (0, 10, 25, or 50 ppm) in eight blocks. In each block, 20 pneumonia-free 16-week old Large White x Landrace gilts were used.

3.2.4 *Ammonia exposure*

The aim of this trial was to investigate the effects of short-term exposure of ammonia at various concentrations, being 0, 10, 25 and 50 ppm ammonia. Ammonia gas in nitrogen was supplied from G size cylinders (at concentrations of 0, 10, 25 and 50 ppm ammonia) (BOC gases Australia). The ammonia gas was pumped into each individual feed bin at a rate of 12 l/min for 30 min while pigs were eating, and each pig was observed to ensure maximum exposure.

Ammonia exposure was based on the fact that ammonia levels fluctuate in pig buildings during a 24 h period. In Australia one of the peaks of ammonia in naturally ventilated sheds with effluent channels occurs when these channels are emptied and flushed with water. Preliminary investigations had shown that levels of ammonia peaked after flushing and remained elevated for up to 60 min. Hence short-term exposure to high levels of ammonia gas appears to be common in naturally ventilated sheds.

The method chosen for ammonia exposure (30 min at feeding) was chosen to simulate exposure to high levels of gas over a short period and to ensure that each pig was exposed for a minimum of 15 min. The current Occupational Safety and Health Administration (OHSA) permissible exposure limit for ammonia is 35 ppm as a 15-min short-term exposure limit (STEL) (OHSA, 1988). A STEL is a 15-min time-weighted average exposure which should not be exceeded at any time during the workday.

Prior testing, using a multi-gas monitoring (MGM) machine developed in-house by Mr Nicholas Masterman, had ensured that the gas entering the feed bin was at the correct concentration. Periodic testing was performed during the trial. A sensor was placed in the top right corner of the feed bin, behind a mesh barrier that prevented interference by the pig. The flow rate chosen ensured that the majority of the gas was inhaled, and did not disperse beyond the feed bin. Prior testing, and periodic testing during the trial, failed to measure ammonia levels between adjacent pens. Short-term measures (1 min) of ammonia concentrations were taken from each of the feed bins during ammonia exposure using an aspirating pump connected to standard colorimetric gas tubes (Kitagawa, Komyo Rikagaku Kogyo, Japan).

3.2.5 *Isolation and classification of bacteria*

The alpha haemolytic cocci (AHC) were obtained using a 6-stage Andersen Sampler (Andersen Instruments Incorporated, Atlanta, USA) loaded with Columbia horse blood agar (HBA) plates (Medvet Diagnostics, Adelaide) from the airspace of a nearby shed housing growing pigs located at the Pig and Poultry Production Institute (PPPI) herd housed in the University of Adelaide, Roseworthy Campus Research Piggery (Farm 1) for five min at a flow rate of 1.9 l/min. Alpha haemolytic colonies with differing colony morphologies were selected from each group of plates for phenotypic identification using API 20 Strep strips used in accordance with the manufacturer's instructions (bioMérieux, La Balme les Grottes, France). Organisms yielding unique API profiles were then sequenced by 16S rRNA gene sequencing. For API profiles shared across multiple isolates, only a single representative isolate was sequenced.

Sequence matches were sought in the GenBank+EMBL+DDBJ+PDB sequences using the program BLASTN ver. 2.2.24+ (Altschul *et al*., 1997). The matching was performed after the GenBank accession date for Aerococcus suis partial 16S rRNA gene (type strain 1821/02T; 29th June 2009). The isolates of VGS were prepared and identified at The University of South Australia, Division of Health Sciences, School of Pharmacy and Medical Sciences and the South Australian Department of Health. The inoculum used was 200,000 cfu/ml.

3.2.6 *Bacterial exposure*

Thirty min after feeding, pigs received an intranasal inoculation of 2 x 10^5 of alpha haemolytic cocci (AHC), administered as 1 ml (0.5 ml in each nostril) solution of AHC suspended in PBS at a concentration of 2 x 10^5 cfu/ml. Sterile saline (0.5 ml in each nostril) was instilled into the nostrils of the designated infection-free (control) pigs. The intranasal solution was delivered as a very fine mist, using a mucosal atomization device (Wolfe Tory Medical Inc, USA), approximately 4 cm into each nostril. The head of the pig was maintained in an elevated position to ensure that the intranasal solution did not discharge from the nostrils.

3.2.7 *Ammonia and carbon dioxide measurement*

Gases such as NH_3 and CO_2 were monitored continuously using a multi-gas monitoring (MGM) machine developed in-house by Dr Nick Masterman. An electrochemical gas monitoring head (Bionics TX-FM/FN, Bionics Instrument Co., Tokyo, Japan) was used to detect internal concentrations of NH_3, and an infrared sensor (GMM12, Vaisala Oy,

Helsinki, Finland) was used to detect CO_2 concentrations. The gas monitoring heads and the supporting electrical components were enclosed in a shock-resistant electrical box. An air delivery system was also built into the MGM machine, which delivered air samples from the sampling points within, and outside, the buildings to the actual gas monitoring heads. Air was drawn at a nominal rate of 0.5 to 0.8 l/min from the sampling points. After each sampling point had been monitored for 15 min, the system was purged for 15 l/min with fresh air drawn from outside the buildings to flush out the sampling lines and zero the NH_3 monitoring head. Electronic (voltage) tags corresponding to the internal and external sampling sites were logged, which enabled automatic separation of the data. A computer program was developed to facilitate the automatic separation and graphing of data. The program also contained algorithms for calculating the amount of time spent above and below the relevant recommended levels. At the end of each data collection period, the raw data were assessed by the data collectors. If drift had occurred in the raw dataset (i.e., if during the purge periods the data did not demonstrate a dramatic decline towards zero in the case of NH_3, or to the expected ambient levels in the case of CO_2), the data were discarded from the dataset designated for analysis. The MGM machine was calibrated frequently using a custom-made 2500 ppm CO_2 mixture and a standard 50 ppm NH_3 calibration gas mixture (Calgaz, Air Liquide Australia, Ltd., Australia). For most monitoring events, the enclosure containing the gas monitoring heads was deployed as close to the actual sampling locations as possible to minimize the length of sampling tube used. Sampling tubes were not heated, as condensation was unlikely to occur under typical Australian climatic conditions. A filter was attached to the end of each intake tube to prevent the

deposition of particles in the sampling line. The sampling lines were thoroughly cleaned both internally and externally using anti-viral disinfectant (Virkon S®, Antec International, Suffolk, UK) to avoid cross-contamination between trials.

3.2.8 *Airborne particle measurement*

Inhalable and respirable particle concentrations were measured using GilAir pumps (Gilian Instrument Corp., West Caldwell, USA) which were connected to cyclone filter heads (for respirable particles) and Seven Hole Sampler (SHS) filter heads (for inhalable particles) (Casella Inc., Kempston, UK) and operated at 1.9 and 2.0 l/min flow rate, respectively. The fibreglass filter papers (Whatman Ltd, USA) were conditioned, following standard operational procedures for gravimetric air sampling (Anon, 1987) by being kept in the laboratory for approximately 24 h before and after deployment. A field blank (matched-weight filter cassesette using filter papers from the same batch used for sampling, with no air drawn through it) was used at each sampling site. Gillian field calibration instrumentation (Gillian Instrument Corp., West Caldwell, N.J. USA) was used to recalibrate the flow rates of the sampling pumps. The pumps were operated over an 8 h period. After sampling, the filter heads were taken back to the laboratory weighing room and the filter paper weighed to the nearest 0.001 milligram using a microbalance (Sartorius MC5, Sartorius AG, Goettingen, Germany) and the respirable and inhalable dust levels were calculated. This protocol was performed twice a week (during the 2 week period), to ensure that the total and respirable dust levels were well below maximum acceptable limits during the trial.

3.2.9 *Bacteria measurements*

Total viable airborne bacteria were measured using an Andersen viable six-stage bacterial impactor (Andersen Instruments Incorporated, Atlanta, USA) loaded with Columbia horse blood agar (HBA) plates (Medvet Diagnostics, Adelaide, Australia). The airspace of the room was sampled for five min at a flow rate of 1.9 l/min at 10 different sites 0.5 m above the floor once a day (morning) to ensure that the total viable airborne bacteria were close to zero. The bacteria plates were incubated for 48 h at 37 ^{O}C and the number of colonies were counted manually on top of a light box. The concentration of viable airborne microorganisms was calculated and expressed as colony forming units (cfu/m^3).

The Andersen sampler was designed to operate at a flow rate of 28.3 l/min (Andersen, 1958), however, the GilAir pumps used in this trial (Gilian Instrument Corp., West Caldwell, USA) could not operate at that level. The flow rate of 1.9 l/min was validated by Arthur Barton, Curtin University and Greg Yarrick, Department of Occupational Health and Safety, Western Australia (Skirrow *et al*., 1995). This methodology was also validated by the Health, Housing and Welfare Group, Primary Industries and Resources of South Australia by collecting multiple air samples consecutively from the same location in several pig sheds and establishing that the accuracy at any specific site was within a 5% margin of error (Report to PRDC: All-in/All-out Production Project, 1999). In this study, the Andersen sampler was not used quantitatively; it was merely used as a collection device for viable organisms that were then amplified. The data was

only used to make comparisons in 'before-and-after' studies and not to make quantitative assessments of the environment.

3.2.10 *Temperature and humidity measurements*

Temperature and humidity data were recorded using Tinytalk temperature and humidity loggers (Tinytalk-2, Hastings Dataloggers Pty. Ltd., Port Macquarie, Australia). Sensors were used to measure both internal and external temperature and humidity. Sensors were located 1.5 m above the floor, which was as close to pig-height level as possible while still precluding interference by the pigs. This protocol was performed to ensure that the computer-controlled room temperature and humidity system was working correctly during the trial.

3.2.11 *Feed intake and weight measurement*

Pigs were fed 1.5 kg of the standard Roseworthy piggery finisher diet (13% protein, 2% fat, 6% fibre, 13MJ/kg digestible energy) at 9:00 am and 1.5 kg at 4:00 pm each day. Orts were weighed, recorded, and discarded. Pigs were weighed once daily at 8:30 am, just prior to their first feed.

3.2.12 *Blood collection from anterior vena cava*

A nose snare was used to restrain the pig while 2 x 10 ml of blood was taken from the anterior vena cava pre- and post- pollutant exposure. Blood was collected in sterile 10 ml glass containers with 10 μl preservative-free heparin per ml of blood. The tubes containing one replicate of the blood were put into a cool, insulated storage box and

were analysed within an hour of being taken. The other replicate of blood samples was put into a cool, insulated storage box and sent via air courier to the Australian Animal Health Laboratories (AAHL), Commonwealth Scientific and Industrial Research Organisation (CSIRO), Geelong within 24 h, where serum was removed and frozen within an hour of being received.

3.2.11 *Phagocytosis assay*

Phagocytic potential was measured by the uptake of microspheres by heterophilic polymorphonuclear leucocytes and adapted from Kato *et al*., (2000). For each sample, 100 µl of heparinised whole blood in 1.9 ml of phosphate buffered saline (PBS) was incubated for 5 min at 37 ^{O}C to equilibrate. Negative control cells were incubated at 4 ^{O}C. Forty µl of a 2.5% suspension of fluorescent 1 µm diameter microspheres (Polysciences, Warrington, PA) in PBS was added to each tube and incubated at 37 ^{O}C continued for a further 60 min. Control tubes were incubated at 4 ^{O}C. The suspension was gently pipetted onto 1 ml of foetal calf serum and centrifuged (1400 rpm for 5 min = 200 G) to generate a cell pellet. The supernatant containing free beads was aspirated and discarded. The cells were resuspended in 1-2 ml PBS+0.1% trypsin (Type IIIs, Sigma) and 5 nMol ethylenediaminetetraacetic acid (EDTA) (i.e. equal parts stock tissue culture Trypsin/Versine) and then incubated at 37 ^{O}C for 10 min to detach adhered but not phagocytosed beads. Once again, the suspension was gently pipetted onto 1 ml foetal calf serum and centrifuged (1400 rpm, 5 mins = 200 G) to generate a cell pellet. The cells were washed twice in cold PBS. To lyse red blood cells (RBCs), 100 µl Optilyse B (for BD flow cytometers) was added to the cell pellets, which were

then mixed thoroughly and incubated for 10 min. 1 ml sterile distilled water was then added, mixed thoroughly and incubated for a further 10 min. The flow cytometric profiles were acquired using a FACSCalibur Flow Cytometer in conjunction with CELLQuest software (BD Biosciences, San Jose, CA). The analysis gate was set around heterophils on forward and side scatter profiles. The results are expressed as the percentage of monocytes and heterophils that contained fluorescent microspheres.

3.2.12 *Lymphocyte proliferation*

The method to measure proliferative response of the lymphocytes was based on the incorporation of thymidine into replicating DNA, and adapted from Maluish and Strong (1986). Peripheral blood mononuclear cells (PBMC) were obtained from venous blood using IsoPrep gradient, according to the manufacturer's recommendations (Robbins Scientific, Sunnyvale, CA). Recovered cells (1 x 10^9 cells/well) were resuspended in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 pg/ml streptomycin, 0.5-1% FS, indomethacin (1.5-6 pg/ml), 5 x 10^{-5} M 2-mercaptoethanol, with or without the stimulant concanavalin A (Sigma-Aldrich, St Louis, MO) for a total volume of 200 μ1 and cultured in 96-well U-bottom plates in a humidified atmosphere of 5% CO_2, at 37 ^{O}C. The day before harvest, 1.0 μCi/well of tritiated-thymidine (Amersham Biosciences, Uppsala, Sweden) was added for the final 18 h at which time the cultures were harvested onto glass filters. Filters were placed into pouches with 5 ml of scintillant, and incorporated tritiated-thymidine activity was determined by a Microbeta Trilux 1450 beta counter (EG&G Wallac, Gaithersburg, MD). All tests were performed as triplicate cultures. Lymphocyte proliferation was

expressed as a stimulation index (SI = mean counts of stimulated cultures/mean counts of medium control).

3.2.13 *Surface staining*

The method to measure cell surface staining of the lymphocyte subsets was adapted from that of Chamorro *et al*., (2000). Cells were incubated at room temperature for 20 min with the appropriate antibody panel conjugated fluorescein isothiocyanate (FITC), phycoerythrin (PE), streptavidin peridinin chlorophyll protein (PerCP)/streptavidin-Cy-Chromme (CyC), or allophycocyanin (APC) (BD Biosciences Pharmingen, San Diego CA). Following immunofluorescent labelling, red blood cells were first lysed with 1 ml fluorescence activated cell sorting (FACS) FACSLyse (BD Biosciences Pharmingen BD, San Diego, CA) for 10 min. The cells were washed with 1 x phosphate buffered saline (PBS) and fixed in 1% formaldehyde. Three- or four-colour flow cytometric analysis was performed using a FACSCalibur Flow Cytometer in conjunction with CELLQuest software (BD Biosciences, San Diego CA). The lymphocyte and monocyte gates were set according to the forward (FSC) and side scatter (SSC) properties of pig leukocytes. Measures of the proportions of lymphocytes expressing the CD4, CD8 or CD21 markers, the proportion expressing both the CD4 and CD8 markers, and the ratio of the CD4 to CD8 markers were obtained. The results were expressed as the mean percentage of cells expressing the particular phenotypic marker.

3.2.14 *Lung pathology*

Pigs were transported to Primo abattoir at Port Wakefield, South Australia. The respiratory tract from each pig was collected in a numbered plastic bag. A section (3 cm^3) was taken from the dorsal diaphragmatic lobe of the right lung, and a section (5 mm wide) was taken from the trachea, just anterior of the bifurcation. The location of the lung and trachea sections had been standardised by the Australian National Pig Health Monitoring Scheme (Jackowiak, 2000). These sections were placed in tissue collection jars filled with 10% buffered formalin solution.

3.2.15 *Tissue fixation, processing, embedding and sectioning*

Samples were washed with phosphate buffered saline (PBS) to remove excess blood. The following ethanol dehydration program was followed: 70% ethanol 60 min, 80% ethanol 60 min, 95% ethanol 30 min, 95% ethanol 90 min, absolute ethanol 120 min, absolute ethanol 120 min, Histolene/ethanol 60 min, Histolene 120 min, Paraffin wax (Histoplast) 120 min. Samples were embedded in paraffin wax using Tissue-Tek II embedding machine (LabTek Division, Miles Lab. Inc, Naperville, Il) and 7 μm sections cut using a microtome (Lietz 1512, Ernst Leitz, Wetzlar). Sections were fixed onto poly-lysine (Sigma Diagnostics, St. Louis) coated slides and oven dried at 60 ^{O}C for up to 24 h prior to staining with hematoxylin and eosin.

3.2.16 *Histopathological examination*

Ten sections, chosen at random, were examined (Olympus BX60 microscope) in each animal to assess the state of the epithelial layer, and the number, percentage and type of

inflammatory and immune cells present. A Leica DC500 camera was used for all histological pictures presented.

3.2.12 *Statistical analyses*

Windows based SPSS 17.0, (SPSS Inc, Chicago, USA, 2009) was used to conduct statistical manipulation of the data. Statistical models were developed using two-way analysis of variance (ANOVA) procedures to test treatment effects between ammonia and alpha haemolytic cocci (AHC). Regressions were analysed manually after Zar (1999). ANOVA was used to determine whether slopes were significantly different from zero, and Student's t-test was used to determine whether two slopes significantly differed from each other. Leucocyte data from before and after inoculation were analysed using paired sample t-tests in Genstat (Lawes Agricultural trust, Rothamstead, United Kingdom).

3.3 Results

3.3.1 *Aerial alpha-haemolytic cocci (AHC)*

Twenty-seven isolates of alpha-haemolytic cocci were characterized. Genotypic identification of unique API 20 STREP phenologies using 16S rRNA gene sequencing identified 21 of the isolates as Aerococcus viridans, three as Streptococcus alactolyticus, one as Streptococcus pluranimalium, one as Vagococcus lutrae. One phenotypic identification as Aerococcus viridans had an unacceptable number of mismatches amongst the 456 base pairs to all GenBank+EMBL+DDBJ+PDB sequences.

3.3.2 *Growth rate, feed utilisation and voluntary food intake*

It had been my intention to use an organism that normally acts as a commensal; however, I detected subclinical impacts on growth and feed utilisation from the inoculation with alpha haemolytic cocci (AHC) in the absence of an insult to the respiratory mucosae arising from the supply of ammonia during feeding.

There was a slight but non-significant (P<0.05) mean decline in the growth rate of pigs exposed to ammonia alone compared with untreated controls (0 ppm ammonia) and the magnitude of this reduction increased as levels of ammonia increased from 10 to 50 ppm (Fig. 3.1). ADG for control pigs was 813 g, compared to an ADG of 773 g for pigs exposed to 50 ppm ammonia. Growth rates were reduced by 1.8, 3.1 and 4.9% when pigs were exposed to 10, 25 and 50 ppm ammonia respectively (Table 3.2).

The reduction in growth rate was potentiated when pigs were exposed to AHC as well as ammonia (Fig. 3.1). The ADG for pigs exposed to 0 ppm ammonia and AHC at 200,000 cfu/ml was 709 g, compared to 430 g when pigs were exposed to 50 ppm ammonia and AHC at 200,000 cfu/ml. Growth rates reduced by 12.8, 26.5, 35.2 and 47.2% compared to controls (no ammonia and no AHC) when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml) respectively (Table 3.2).

Similar reductions were also evident in feed efficiency (Fig. 3.2); FCR for control pigs was 3.26 compared to 3.33 for pigs exposed to 50 ppm ammonia only. Feed conversion efficiency reduced by 1.3, 0.4 and 2.1% compared to controls when pigs were exposed to 10, 25 and 50 ppm ammonia respectively. FCR for pigs exposed to AHC only was

3.51 and 4.67 for pigs exposed to both 50 ppm ammonia and AHC. Feed conversion efficiency was reduced by 7.4, 16.0, 20.0 and 30.2% compared to controls (no ammonia and no AHC) when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml) respectively (Table 3.3).

There was a slight, but non-significant (P<0.05) mean decline in feed intake in pigs exposed to ammonia compared with untreated controls (0 ppm ammonia) and the reduction increased as levels of ammonia increased from 10 to 50 ppm (Fig. 3.3). The reduction in feed intake was further increased when pigs were also exposed to AHC. There was a reduction in feed intake of 0.8, 4.2 and 2.7% compared to controls when pigs were exposed to 10, 25 and 50 ppm ammonia respectively. There was a reduction in feed intake of 6.9, 13.0, 20.6 and 25.6% compared to controls (no ammonia and no AHC) when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml) respectively (Table 3.4).

Two of the three growth and feed utilisation parameters (VFI, F = 51.4, $F_{0.001(2)1,38} \cong$ 14.5; and ADG, F = 10.4; $F_{0.01(2)1,38} \cong$ 8.9) were significantly affected by inoculation (Table 3.1) compared with uninoculated controls to extents that would be economically important. However, FCR was not significantly affected (F = 2.6).

Table 3.1: Mean growth rate and feed utilisation parameters of gilts inoculated intranasally with 2 x 10^5 cfu of alpha haemolytic cocci (AHC). Pigs offered 3.0 kg/day. VFI – voluntary food intake; ADG - average daily gain; FCR - feed conversion ratio. Data are mean values ± SD. N = 40.

	Control	AHC
VFI (kg /day)	2.50 ± 0.10^{a}	2.32 ± 0.06^{b}
ADG (g liveweight gain /day)	813 ± 97^{c}	709 ± 106^{d}
FCR (kg feed/kg liveweight gain)	3.27 ± 0.38	3.52 ± 0.59

[a, b]Means within a row with different superscripts significantly differ ($P<0.001$)

[c, d]Means within a row with different superscripts significantly differ ($P<0.01$)

While there was little, or no effect of ammonia alone on growth and feed utilisation parameters, the data suggested that a larger sample size might have revealed a decrease in VFI (Fig. 3.6, control slope from zero $F = 5.14$, $F_{0.05(2),1,78} \cong 5.22$). However, ADG (Fig. 3.4, $F = 2.00$) and FCR (Fig. 3.5, $F = 0.15$) were not affected.

The combined impact of ammonia and AHC impacted growth rate and feed utilisation parameters adversely and, with respect to ammonia, in a dose-dependent manner. The differences between the slopes of the regressions of the control animals against ammonia, and the inoculated animals against ammonia, were significant for all three parameters (VFI, Fig. 3.6, $t = 3.79$; ADG, Fig. 3.4, $t = 3.62$; FCR, Fig. 3.5, $t = 3.41$; $t_{0.001(2),156} \cong 3.35$).

Table 3.2: The mean growth rate (average daily gain (ADG)) in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 concentration (ppm)	**ADG (g/day)**	
	NH_3 – B	**NH_3 + B**
0	813 ± 21.6^{a}	709 ± 23.7^{b}
10	798 ± 21.7^{a}	598 ± 18.6^{c}
25	788 ± 18.9^{a}	527 ± 21.9^{cd}
50	773 ± 19.2^{a}	430 ± 20.3^{e}

Differing superscripts indicate a significant difference ($P<0.05$)

Figure 3.1: Mean average daily gain (ADG) in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant ($P<0.05$) between treatment groups.

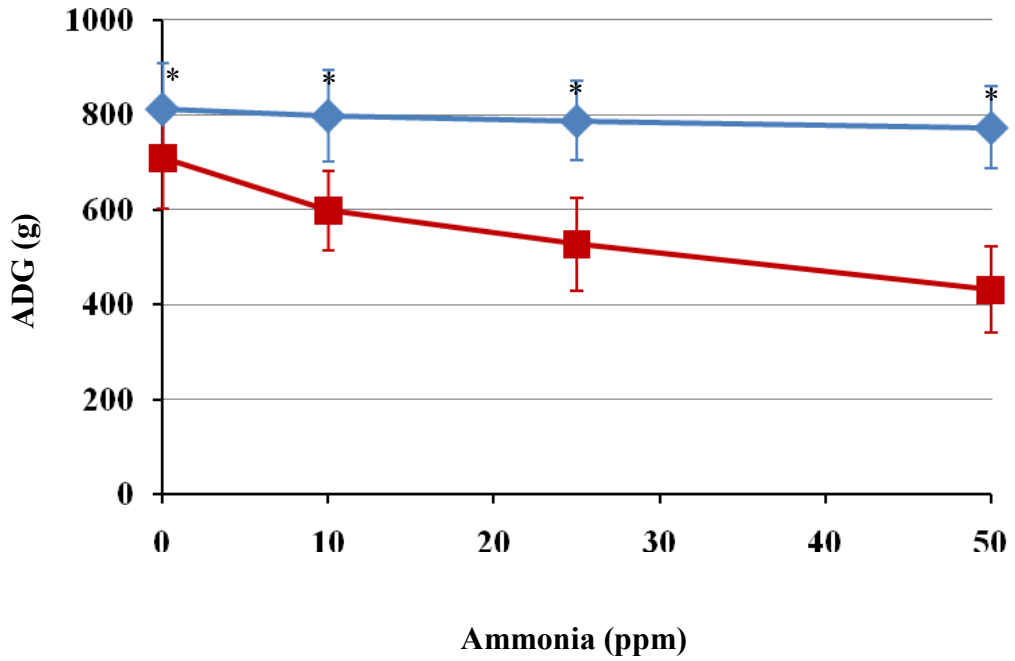


Table 3.3: The mean feed efficiency (FCR) in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 concentration (ppm)	**FCR feed eaten (kg)/kg weight gain**	
	NH_3 – B	**NH_3 + B**
0	3.26 ± 0.08^{a}	3.52 ± 0.13^{a}
10	3.30 ± 0.09^{a}	3.88 ± 0.14^{b}
25	3.27 ± 0.06^{a}	4.07 ± 0.19^{bc}
50	3.33 ± 0.08^{a}	4.67 ± 0.24^{c}

Differing superscripts indicate a significant difference ($P<0.05$)

Figure 3.2: Mean food conversion ratio (FCR) in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant ($P<0.05$) between treatment groups.

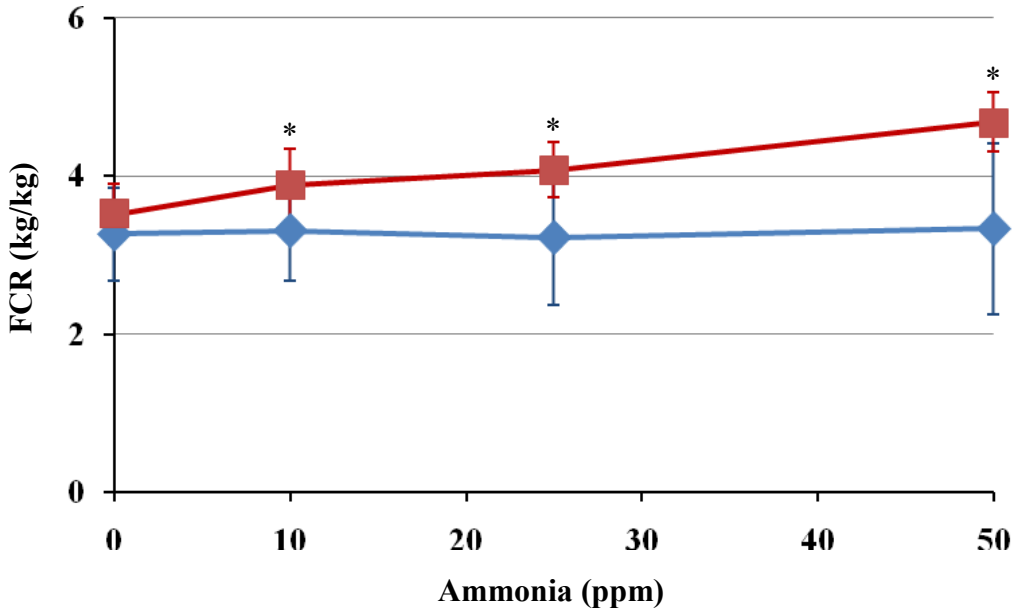


Table 3.4: The mean daily voluntary feed intake (VFI) (kg) in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 concentration (ppm)	Feed intake (g/day)	
	NH_3 – B	NH_3 + B
0	2.62 ± 0.02^{a}	2.44 ± 0.01^{b}
10	2.60 ± 0.03^{a}	2.28 ± 0.03^{c}
25	2.51 ± 0.03^{a}	2.08 ± 0.04^{cd}
50	2.55 ± 0.02^{a}	1.95 ± 0.06^{e}

Differing superscripts indicate a significant difference (P<0.05)

Figure 3.3: Mean daily voluntary food intake (VFI) in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

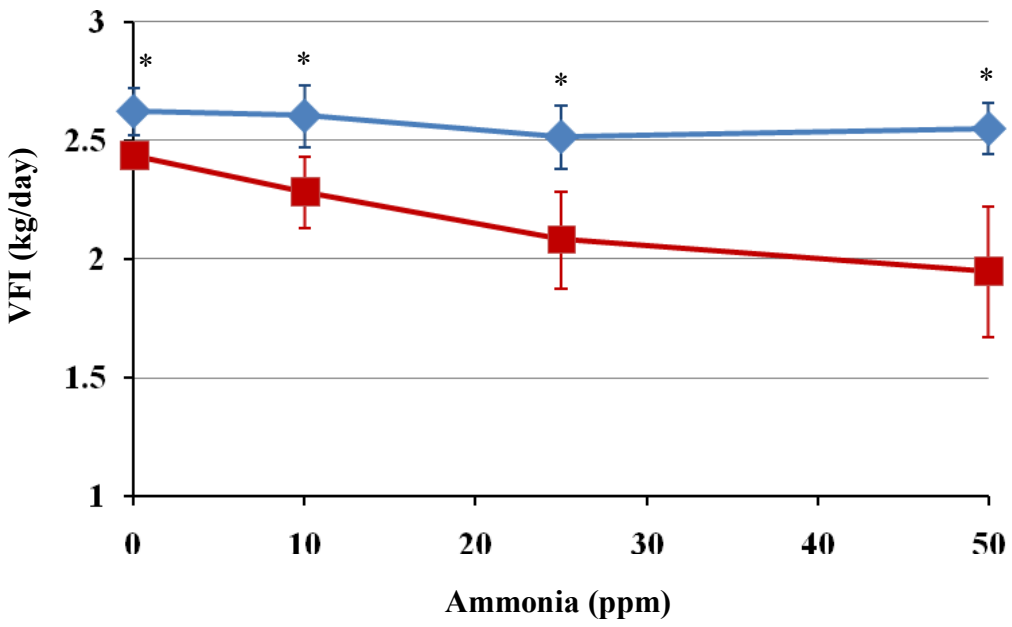


Figure 3.4: Regression graphs for average daily gain (ADG) in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

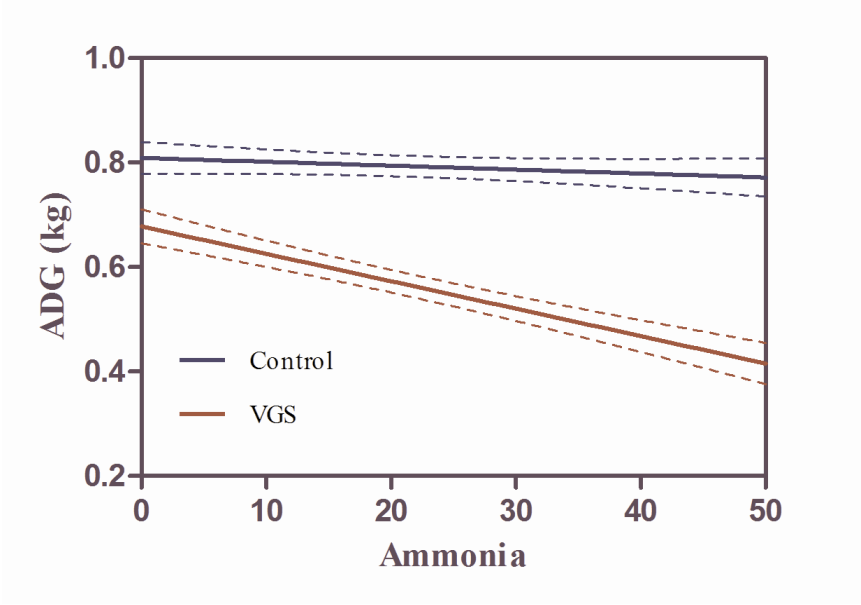


Figure 3.5: Regression graphs for feed efficiency (FCR) in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

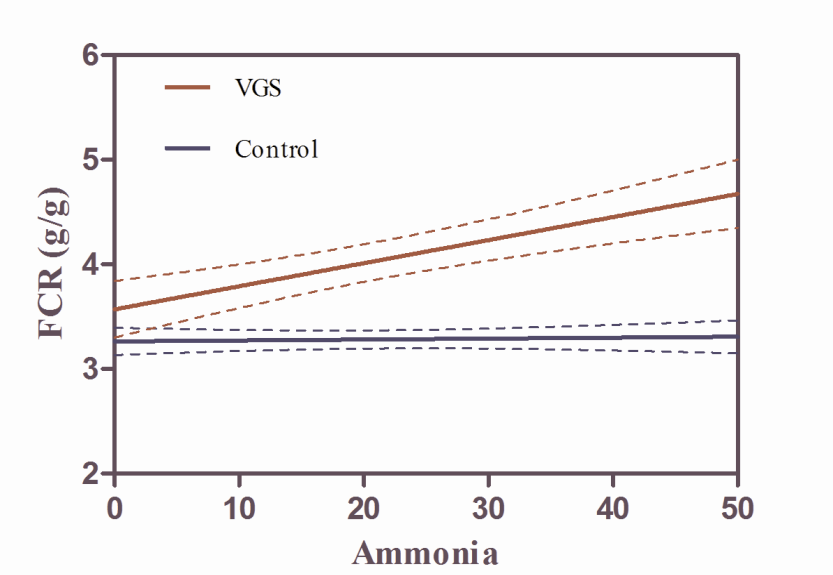
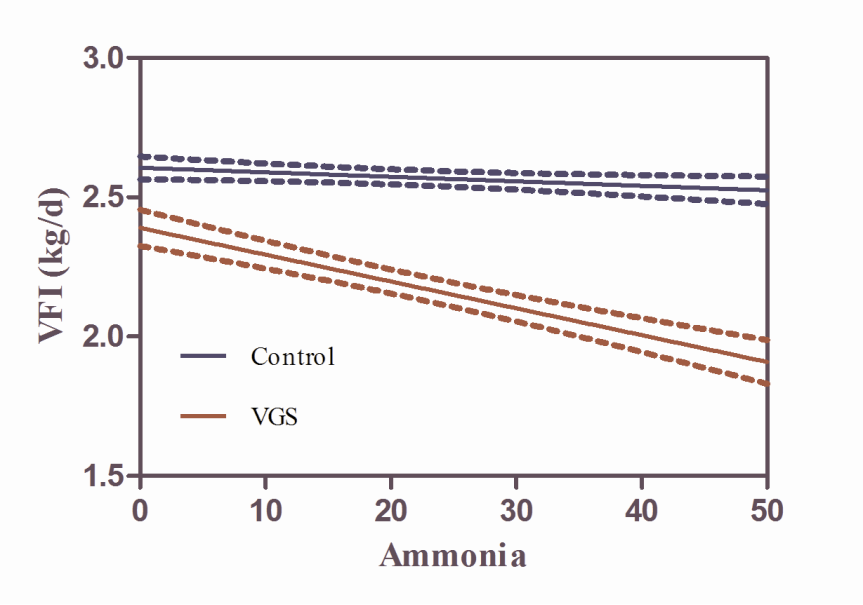


Figure 3.6: Regression graphs for voluntary feed intake (VFI) in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.



3.3.3 *Immune responses*

In addition to the subclinical effects on growth rates and feed utilisation, I detected immune system activation in AHC pigs, indicated by proliferation of T lymphocytes (Table 3.6), an increase in the phagocytic potential of heterophils (Table 3.7), an increase in the proportion of lymphocytes expressing the CD4 marker (Table 3.9) and an increase in the ratio of lymphocytes expressing the CD4 marker to those expressing the CD 8 marker (Table 3.11). Changes were observed in the proportion of lymphocytes expressing the CD21 (Table 3.8) and CD8 marker (Table 3.10). These results are summarised in Table 3.5.

Although lymphocyte stimulation index (LSI) was not consistently increased in pigs exposed to ammonia alone, a significant increase (P<0.05) was evident in pigs exposed to ammonia and AHC. In the latter groups, the stimulation index increased as the concentration of ammonia increased (Table 3.6). The lymphocyte proliferation increased by 16, 16, 17 and 25% during the 14 day trial period when pigs were exposed to 0, 10, 25 and 50 ppm ammonia respectively. Lymphocyte proliferation increased by 31, 49, 67 and 76% compared to controls (no ammonia and no AHC) when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml) respectively (Fig. 3.7).

There was a slight, but non-significant (P<0.05) mean increase in heterophil phagocytic potential (HPP) in pigs exposed to ammonia alone at concentrations of 0, 10 and 25 ppm. A slight, but significant (P<0.05), increase in HPP was observed in pigs receiving 50 ppm ammonia alone during the 14 day trial (Table 3.7). HPP increased 17, 17, 20 and 20% during the 14 day trial period when pigs were exposed to 0, 10, 25 and 50 ppm ammonia respectively. HPP activity increased by 46, 60, 71 and 78% compared to controls (no ammonia and no AHC) when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml) respectively (Fig. 3.8).

Slight, and inconsistent, changes were observed in the proportion of leucocytes expressing the CD21 marker (Table 3.8). There was a significant difference (P<0.05) between pigs receiving 10 and 25 ppm ammonia plus AHC (200,000 cfu/ml), compared to controls (Fig. 3.11).

There were no significant differences (P<0.05) in the proportion of leucocytes expressing the CD4 marker in pigs exposed to ammonia alone at concentrations of 0, 10, 25 and 50 ppm during the 14 day trial (Table 3.9). The proportion of leucocytes expressing the CD4 marker increased significantly (P<0.05) when pigs were exposed to ammonia and AHC (200,000 cfu/ml) (Fig. 3.12). In the latter group, the stimulation index increased as the concentration of ammonia increased from 0 to 50 ppm.

Slight, and inconsistent, changes were observed in the proportion of leucocytes expressing the CD8 marker (Table 3.10). There was a significant difference (P<0.05) between pigs receiving 0 and 50 ppm ammonia plus AHC (200,000 cfu/ml), compared to controls (Fig. 3.15).

There were no significant differences (P<0.05) in the proportion of lymphocytes expressing the CD4 marker to those expressing the CD8 marker (CD4:CD8 ratio) in pigs exposed to ammonia alone at concentrations of 0, 10, 25 and 50 ppm during the 14 day trial (Table 3.11). The proportion of lymphocytes expressing the CD4 marker to those expressing the CD8 marker increased significantly (P<0.05) when pigs were exposed to ammonia and AHC (200,000 cfu/ml) (Fig. 3.16). In the latter group, the stimulation index increased at the concentration of ammonia increased from 0 to 50 ppm.

The effects of AHC were confounded to some extent by activation that was detected in control pigs (no AHC and no ammonia) in which there were significant increases in

both the LSI (Fig. 3.4, $t = 4.89$; $t_{0.001(2),19} = 3.88$) and HPP (Fig. 3.5, $t = 4.65$) over the 14 days of the trial, but there were also significant increases in these parameters in the inoculated pigs (AHC but no ammonia; LSI, $t = 4.35$; HPP, $t = 11.18$), and the magnitudes of the increases in these parameters were significantly greater in the inoculated pigs compared with the control pigs (LSI, $F = 34.3$; HPP, $F = 79.9$; $F_{0.001(2),1,38} \cong 14.5$).

No increases in the proportions of lymphocytes expressing surface markers were detected in the control pigs over the duration of the trial, but significant increases in the proportions expressing the CD4 (Fig. 3.7, $t = 5.53$; $t_{0.001(2),19} = 3.88$) and CD21 (Fig. 3.6, $t = 2.66$; $t_{0.05(2),19} = 2.09$) markers occurred in the inoculated pigs. The magnitude of the increase in the proportion of leucocytes expressing the CD4 marker ($F = 31.6$) was significantly greater in the inoculated pigs compared with the control pigs, and although no change in the CD4:CD8 ratio ($t = 0.95$) was detected in the inoculated pigs over the duration of the trial, the data suggested that a larger sample size would have revealed a difference ($F = 3.34$, exact $P = 0.075$).

When the inoculated pigs were compared with the control pigs at the end of the trial, the LSI ($F = 8.3$; $F_{0.05(2)1,38} \cong 5.5$) and HPP ($F = 43.1$; $F_{0.001(2)1,38} \cong 14.5$) and the CD4 ($F = 41.6$) and CD8 ($F = 9.2$; $F_{0.01(2)1,38} \cong 8.9$) markers were significantly elevated in the inoculated pigs, as was the CD4:CD8 ratio ($F = 8.2$).

Table 3.5: Levels of leucocyte activation before inoculation with alpha haemolytic cocci (AHC) and 14 days after inoculation. Means ± SD. N = 40.

	Control		AHC	
	Before	After	Before	After
Lymphocyte stimulation index	38.9±20.8[a]	46.4±23.2[be]	45.2±12.1[a]	65.6±18.3[bf]
Heterophil phagocytosis (%)	9.3±4.6[a]	11.3±5.5[bg]	15.3±5.8[a]	28.3±10.2[bh]
CD4 positive cells (%)	15.0±2.0	14.8±2.0[g]	15.5±2.7[a]	23.3±5.4[bh]
CD8 positive cells (%)	21.2±4.3	20.7±3.8[i]	23.2±5.3	24.6±6.4[j]
CD4:CD8 ratio	0.76±0.15	0.74±0.16[i]	0.74±0.23	1.00±0.39[j]
CD21 positive cells (%)	12.4±4.3	12.7±3.8	10.6±2.8[c]	11.7±3.2[d]

[a,b]For each treatment, means within a row with different superscripts significantly differ (P <0.001)

[c,d]For each treatment, means within a row with different superscripts significantly differ (P <0.05)

[e,f]Between treatments, means within a row with different superscripts significantly differ (P <0.05)

[g,h]Between treatments, means within a row with different superscripts significantly differ (P <0.001)

[ij]Between treatments, means within a row with different superscripts significantly differ (P <0.01)

Comparison of the pigs exposed to differing concentrations of ammonia in the absence of inoculation with AHC revealed slight, but significant (P<0.05), progressive impacts of ammonia on HPP (Fig. 3.10, control slope from zero F = 12.11, $F_{0.01(2),1,78} \cong 8.33$) and CD4 lymphocyte activation (Fig. 3.14, F = 6.83, $F_{0.05(2),1,78} \cong 5.22$). No significant impacts of ammonia alone on lymphocyte proliferation (Fig. 3.9, F = 0.13), the CD4:CD8 ratio (Fig. 3.18, F = 1.79), or CD21 lymphocyte activation (Fig. 3.13, F = 0.10) were observed, but the data suggested that a larger sample size would have revealed an increase in CD8 lymphocyte activation (Fig. 3.17, F = 3.12, exact P = 0.082).

The combined impact of ammonia at increasing concentrations together with an initial intranasal inoculation of AHC progressively stimulated heterocyte phagocytosis (Fig. 3.10, between-slopes $t = 2.98$, $t_{0.01(2),156} \cong 2.61$), lymphocyte proliferation (Fig. 3.9, $t = 5.56$, $t_{0.001(2),156} \cong 3.35$), and CD4 expression (Fig. 3.14, $t = 31.64$). No impact on the proportion of leucocytes expressing the CD8 marker (Fig. 3.17, $t = 0.31$) was detected, nor on the CD4:CD8 ratio (Fig. 3.18, $t = 1.22$), nor on the proportion of leucocytes expressing the CD21 marker (Fig. 3.13, $t = 1.01$).

Table 3.6: The mean lymphocyte stimulation index (LSI) pre- and post- pollutant exposure in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 - B conc. (ppm)	LSI		NH_3 + B conc. (ppm)	LSI	
	before	after		Before	after
0	38.90 ± 4.66^a	46.40 ± 5.19^a	0	45.20 ± 2.71^a	65.50 ± 4.09^c
10	47.40 ± 5.31^a	56.30 ± 6.96^b	10	47.00 ± 3.48^a	92.10 ± 7.15^d
25	38.80 ± 5.34^a	46.60 ± 6.23^a	25	50.60 ± 3.03^a	152.10 ± 8.23^e
50	35.50 ± 4.97^a	47.40 ± 5.89^a	50	42.80 ± 2.99^a	178.00 ± 13.37^f

Differing superscripts indicate a significant difference ($P<0.05$)

Figure 3.7: Mean lymphocyte stimulation index (LSI) in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

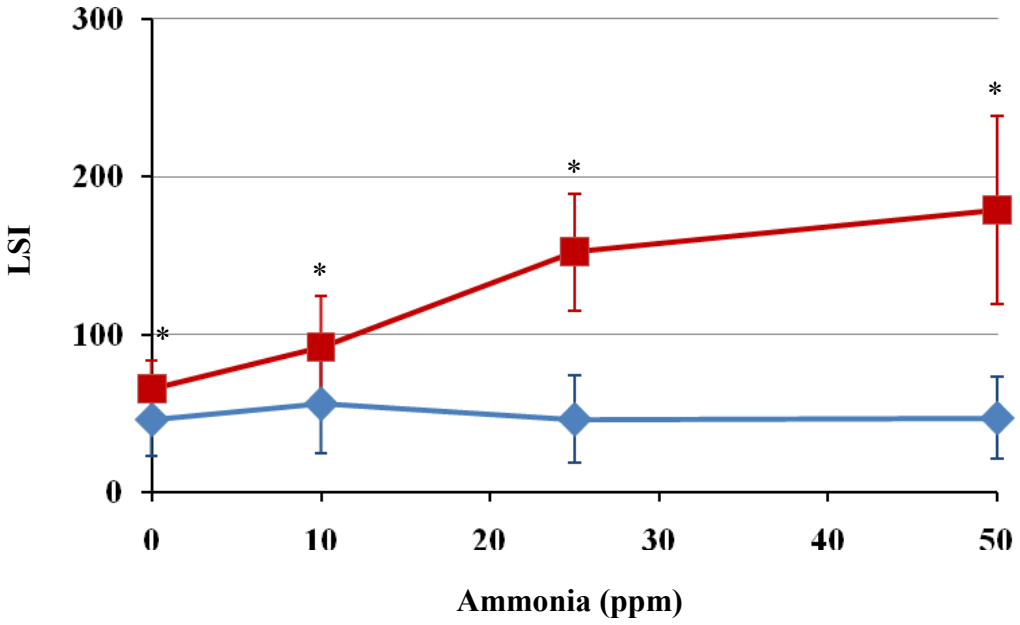


Table 3.7: The mean heterophil phagocytic potential (HPP) pre- and post- pollutant exposure in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 - B conc. (ppm)	HPP		NH_3 + B conc. (ppm)	HPP	
	before	after		Before	after
0	9.30 ± 1.03^{a}	11.25 ± 1.22^{a}	0	15.25 ± 1.30^{b}	28.25 ± 2.28^{d}
10	10.85 ± 0.81^{a}	13.10 ± 0.99^{a}	10	13.30 ± 0.80^{a}	33.20 ± 2.34^{d}
25	10.10 ± 1.07^{a}	12.65 ± 1.62^{a}	25	14.55 ± 0.94^{b}	51.00 ± 3.24^{e}
50	11.75 ± 1.04^{a}	14.63 ± 1.55^{b}	50	14.65 ± 0.96^{b}	66.50 ± 4.47^{f}

Differing superscripts indicate a significant difference (P<0.05)

Figure 3.8: Mean heterophil phagocytic potential (HPP) in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

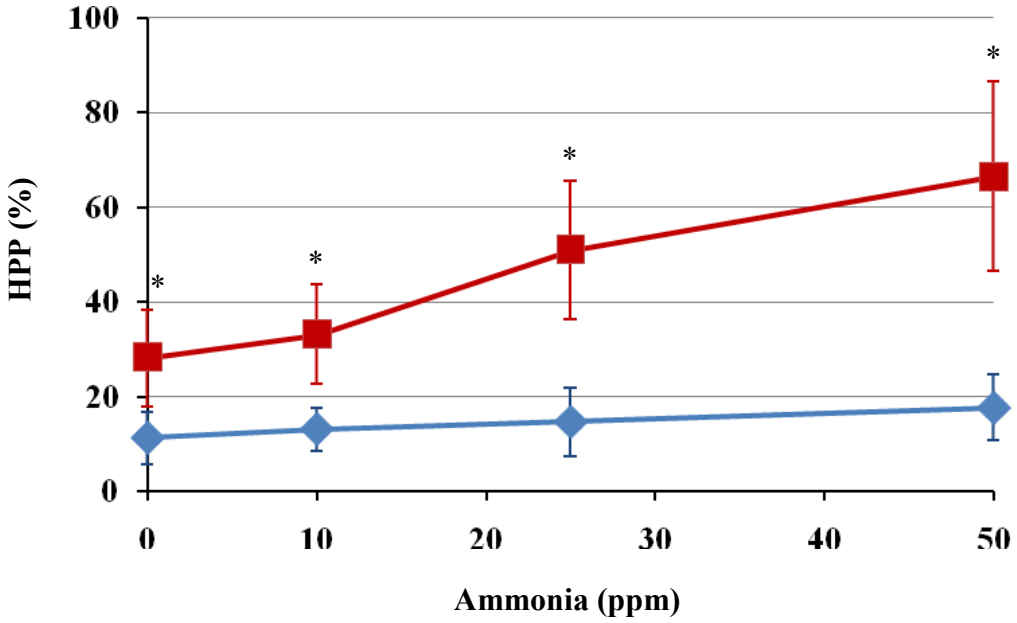


Figure 3.9: Regression graphs for lymphocyte stimulation index (LSI) in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

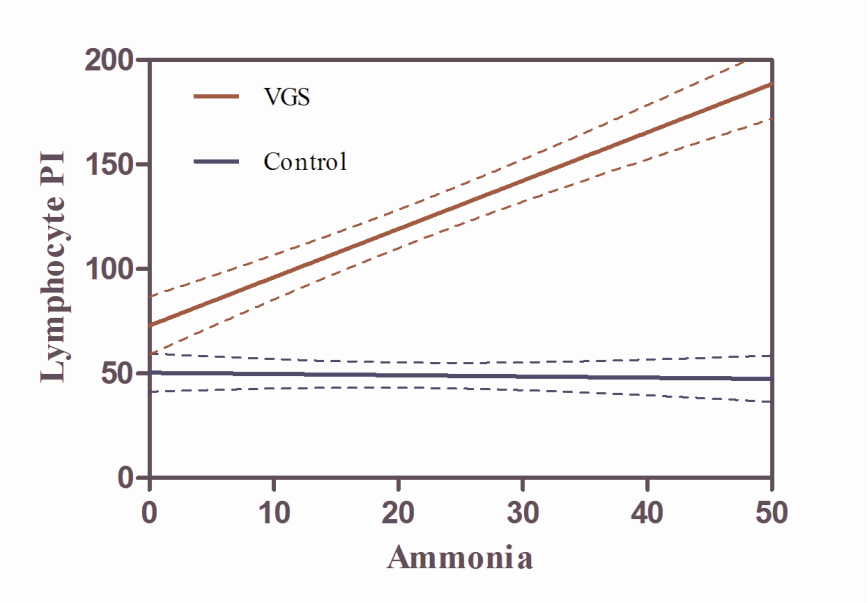


Figure 3.10: Regression graphs for heterophil phagocytic potential (HPP) in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

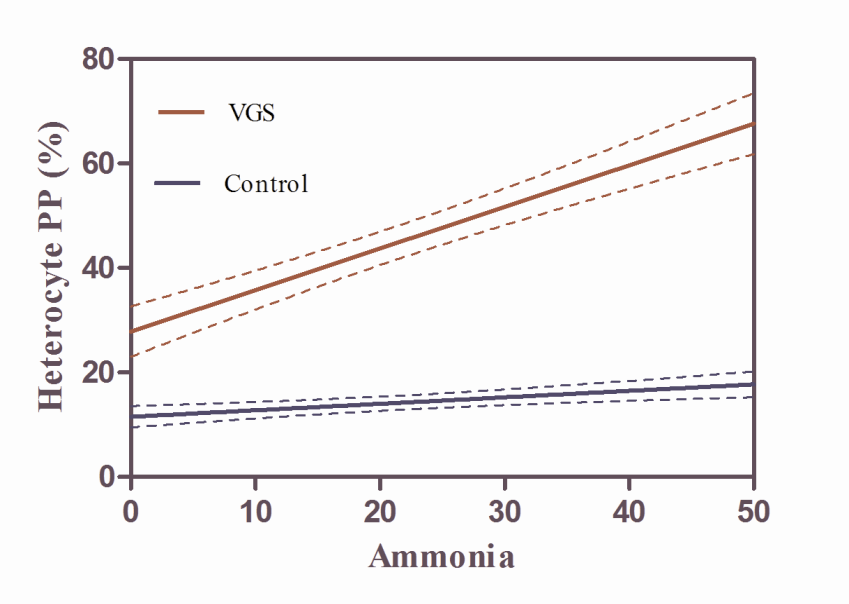


Table 3.8: The mean proportion of lymphocytes expressing CD21 marker pre- and post- pollutant exposure in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 - B conc. (ppm)	LSI		NH_3 + B conc. (ppm)	LSI	
	before	after		Before	after
0	12.39 ± 0.97[a]	12.67 ± 0.85[a]	0	10.56 ± 0.63[a]	11.68 ± 0.72[a]
10	11.96 ± 0.53[a]	12.65 ± 0.48[a]	10	8.89 ± 0.73[a]	10.19 ± 0.68[b]
25	12.70 ± 0.80[a]	13.23 ± 0.72[a]	25	7.45 ± 0.85[a]	9.27 ± 0.60[b]
50	11.67 ± 0.42[a]	12.67 ± 0.50[a]	50	11.55 ± 0.85[a]	12.82 ± 0.66[a]

Differing superscripts within a row indicate a significant difference (P<0.05)

Figure 3.11: Mean proportion of lymphocytes expressing CD21 marker in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

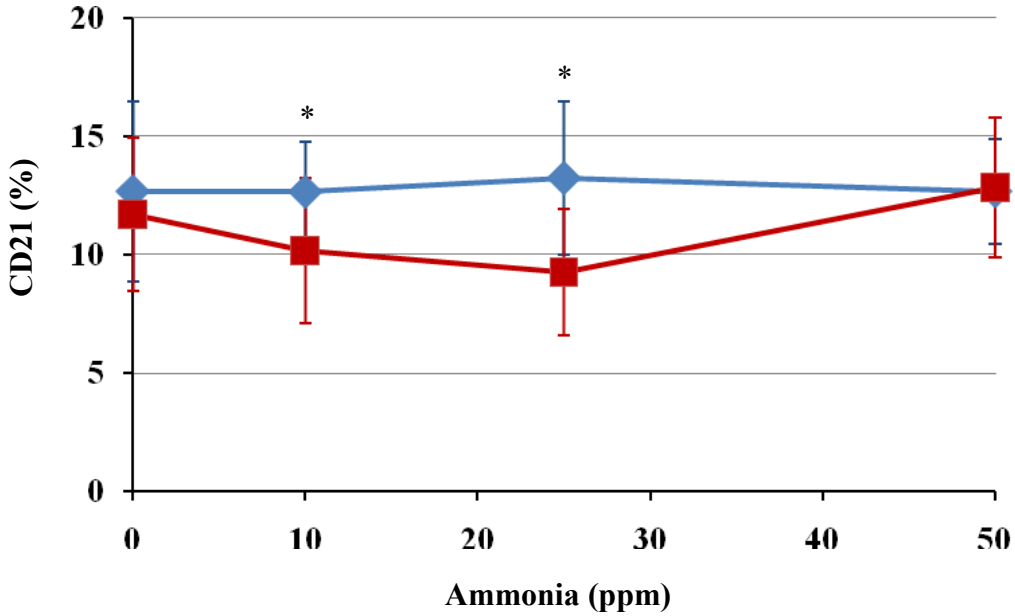


Table 3.9: The mean proportion of lymphocytes expressing CD4 marker pre- and post-pollutant exposure in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 - B conc. (ppm)	LSI		NH_3 + B conc. (ppm)	LSI	
	before	after		Before	after
0	15.03 ± 0.45^a	14.78 ± 0.45^a	0	15.54 ± 0.61^a	23.30 ± 1.24^b
10	15.07 ± 0.51^a	15.39 ± 0.61^a	10	16.11 ± 0.61^a	22.43 ± 0.88^b
25	14.59 ± 0.74^a	15.51 ± 0.60^a	25	15.79 ± 0.60^a	29.72 ± 1.624^c
50	16.03 ± 0.50^a	16.77 ± 0.54^a	50	18.05 ± 0.76^a	33.32 ± 1.18^c

Differing superscripts indicate a significant difference (P<0.05)

Figure 3.12: Mean proportion of lymphocytes expressing CD4 marker in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

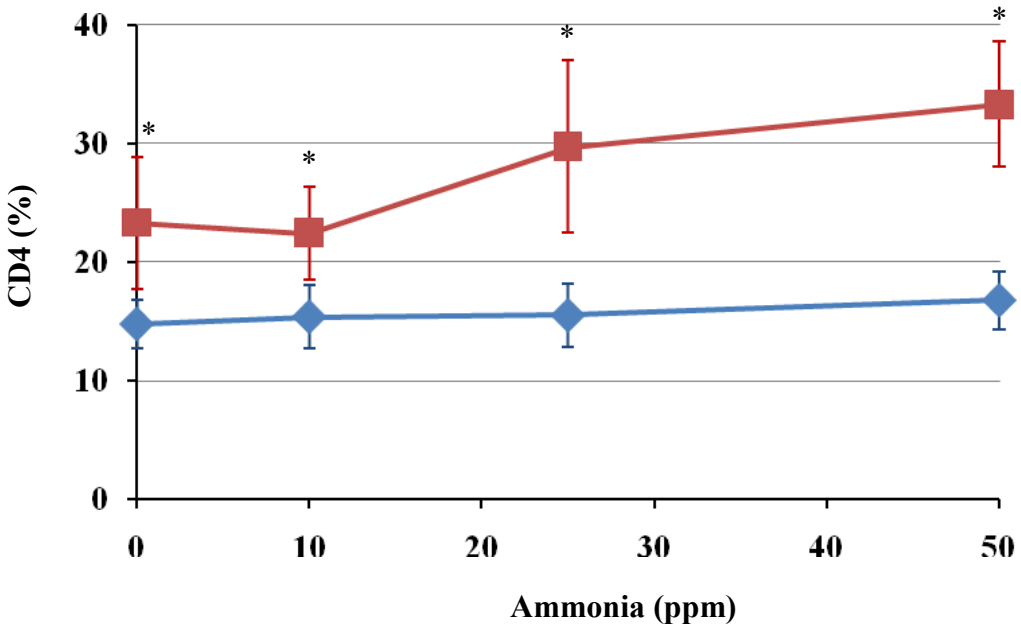


Figure 3.13: Regression graphs for proportion of lymphocytes expressing CD21 marker in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

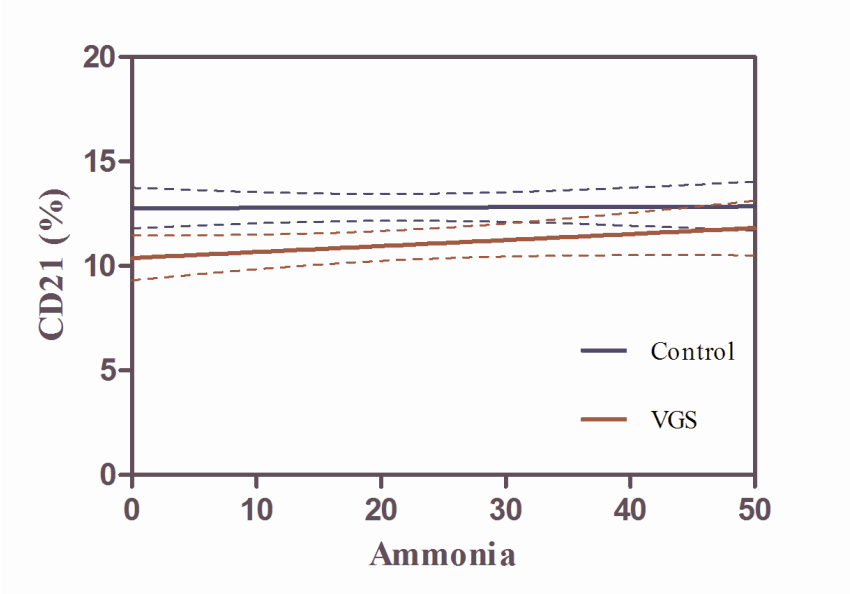


Figure 3.14: Regression graphs for proportion of lymphocytes expressing CD4 marker in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

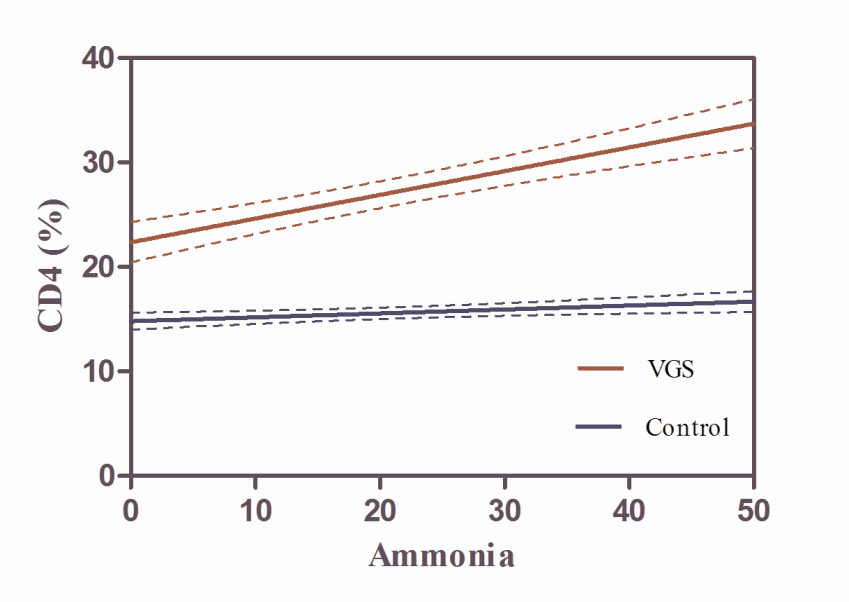


Table 3.10: The mean proportion of lymphocytes expressing CD8 marker pre- and post- pollutant exposure in pigs receiving ammonia by itself (NH_3 - B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 - B conc. (ppm)	LSI		NH_3 + B conc. (ppm)	LSI	
	before	after		Before	after
0	20.18 ± 0.85^a	20.65 ± 0.84^a	0	22.16 ± 1.17^a	24.56 ± 0.98^b
10	20.65 ± 1.28^a	24.70 ± 1.11^b	10	23.02 ± 1.09^a	23.20 ± 0.96^a
25	19.13 ± 0.97^a	24.97 ± 1.30^b	25	19.98 ± 0.85^a	25.33 ± 1.03^a
50	22.65 ± 0.99^a	22.82 ± 1.27^a	50	23.27 ± 0.77^a	26.67 ± 1.20^b

Differing superscripts indicate a significant difference ($P<0.05$)

Figure 3.15: Mean proportion of lymphocytes expressing CD8 marker in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

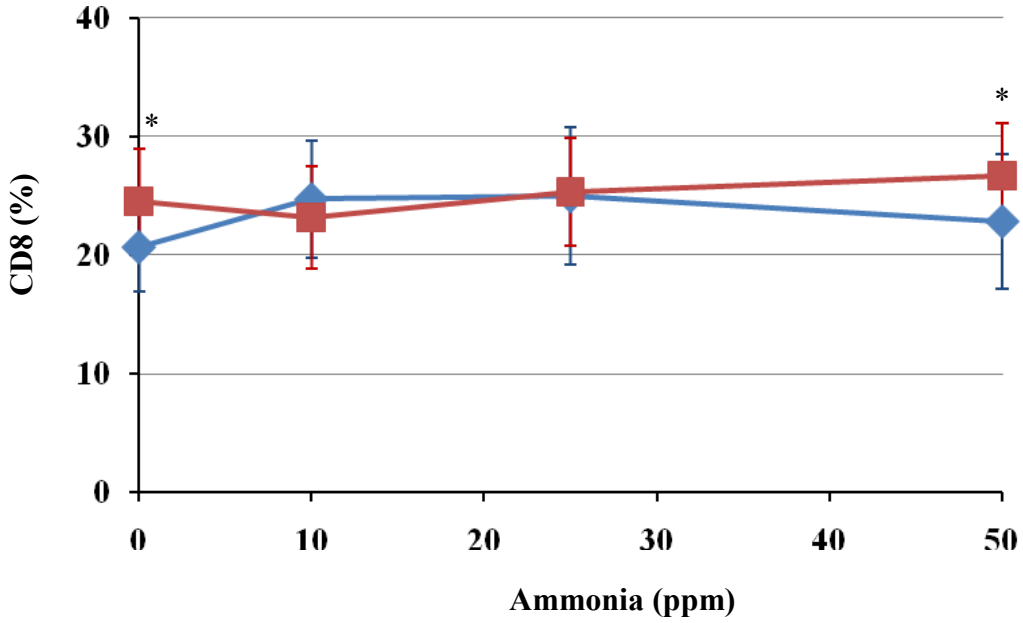


Table 3.11: The mean CD4:CD8 ratio, ratio of lymphocytes expressing the CD4 marker to those expressing the CD8 marker in pigs receiving ammonia by itself (NH_3 – B) or ammonia and alpha haemolytic cocci (AHC) (NH_3 + B). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SEM.

NH_3 - B conc. (ppm)	LSI		NH_3 + B conc. (ppm)	LSI	
	before	after		Before	after
0	0.76 ± 0.03[a]	0.74 ± 0.04[a]	0	0.74 ± 0.05[a]	1.00 ± 0.09[b]
10	0.79 ± 0.06[a]	0.65 ± 0.04[a]	10	0.72 ± 1.03[a]	1.02 ± 0.09[b]
25	0.78 ± 0.05[a]	0.66 ± 0.05[a]	25	0.81 ± 0.04[a]	1.24 ± 0.13[bc]
50	0.73 ± 0.03[a]	0.80 ± 0.07[a]	50	0.78 ± 0.01[a]	1.27 ± 0.04[c]

Differing superscripts indicate a significant difference (P<0.05)

Figure 3.16: Mean CD4:CD8 ratio, proportion of activated CD4+ and CD8+ markers on T lymphocytes in pigs receiving ammonia by itself (◆) or ammonia and alpha haemolytic cocci (AHC) (■). AHC concentration was 200,000 cfu/ml. N = 160. Data are mean values ± SD. *Statistically significant (P<0.05) between treatment groups.

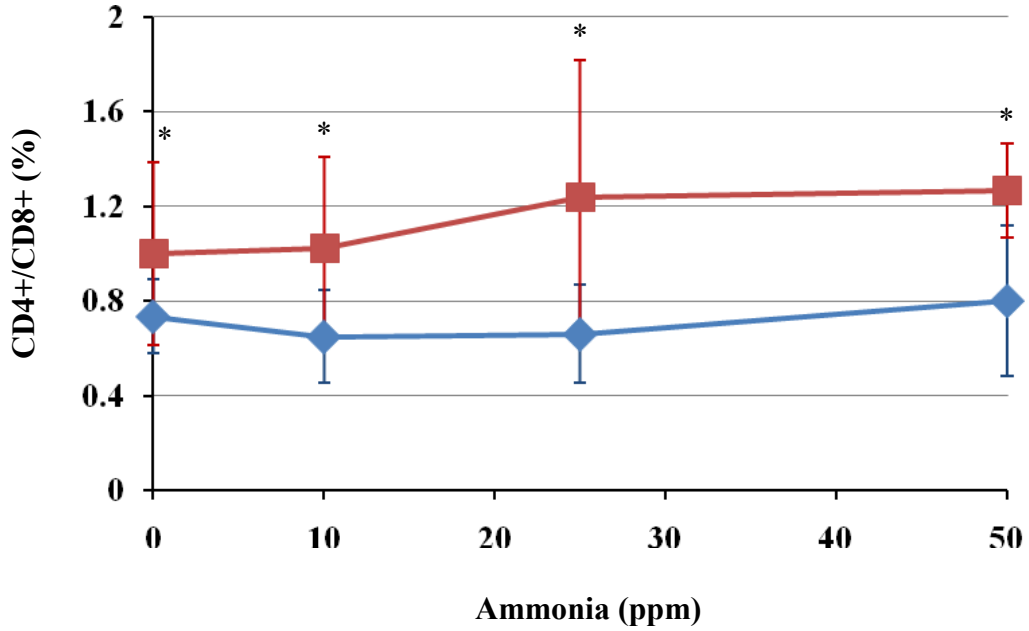


Figure 3.17: Regression graphs for proportion of lymphocytes expressing CD8 marker in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.

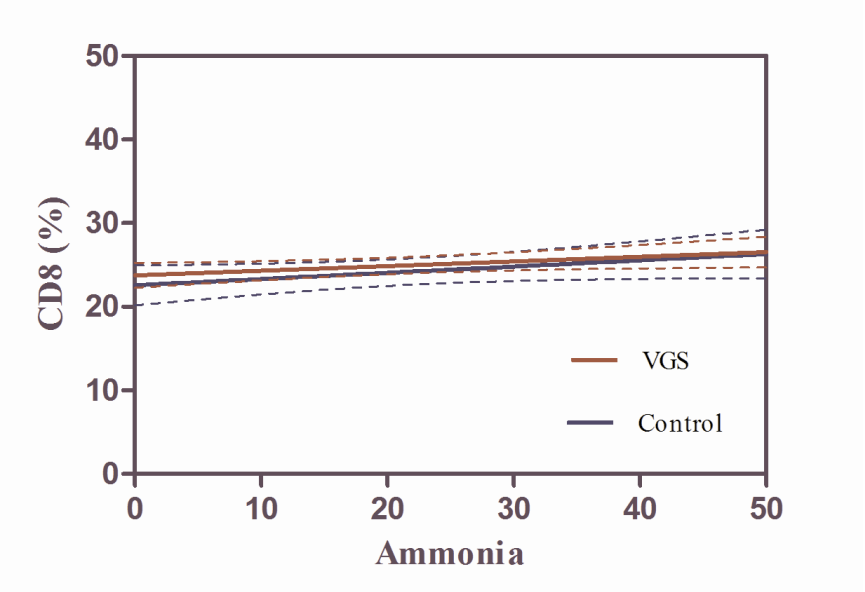
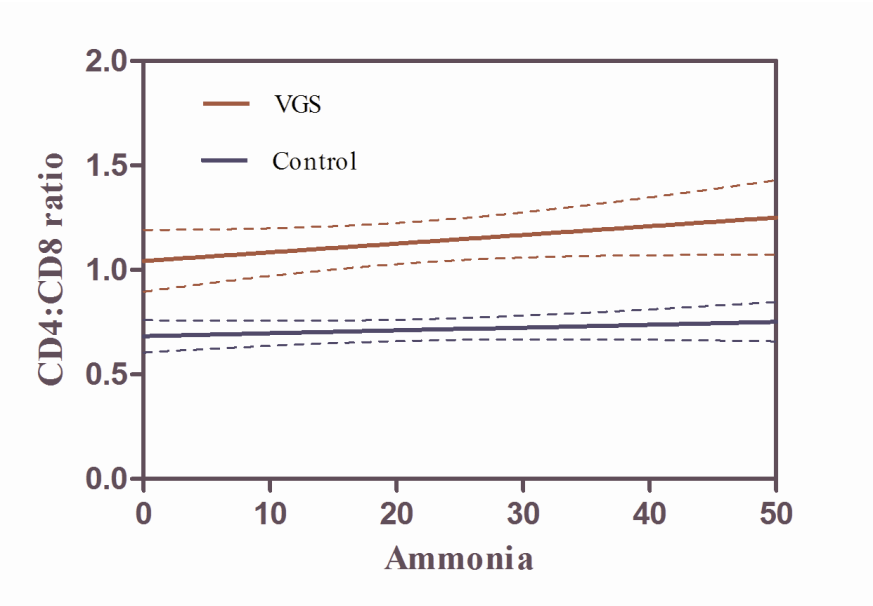


Figure 3.18: Regression graphs for the ratio of lymphocytes expressing the CD4 marker to those expressing the CD8 marker in pigs receiving ammonia by itself (—) or ammonia and alpha haemolytic cocci (AHC) (—). AHC concentration was 200,000 cfu/ml. 95% confidence intervals shown. N = 160.



3.3.4 *Gross pathology*

Macroscopic lesions were not observed in any of the lungs examined at slaughter, regardless of pre-slaughter treatment. Lungs were free of consolidation and there was no evidence of pleurisy. Airways appeared free, but varying degrees of mucus were noted.

3.3.5 *Microscopic changes in lung tissue*

A mild to severe alveolitis dominated by mononuclear leucocytes was present in tissue sections of lungs collected from pigs exposed to ammonia with the cellular reaction increasing as the concentration of ammonia increased. Similar changes were observed

in lung sections taken from pigs exposed to both ammonia and AHC, but in addition, peribronchial foci of monocytic inflammatory cells were observed, especially at the higher concentrations of ammonia. The photos appearing on pages 129 to 136 are not of the same structure of the respiratory tract. They are provided as examples of changes observed in the respiratory tissue and not intended to provide more than a visual example of changes noted.

Figure 3.19: Histopathology slides of control pig lung exposed to 0 ppm ammonia, at (top to bottom) 10x, 20x and 40x magnification. Scale bar represent 100 μm.

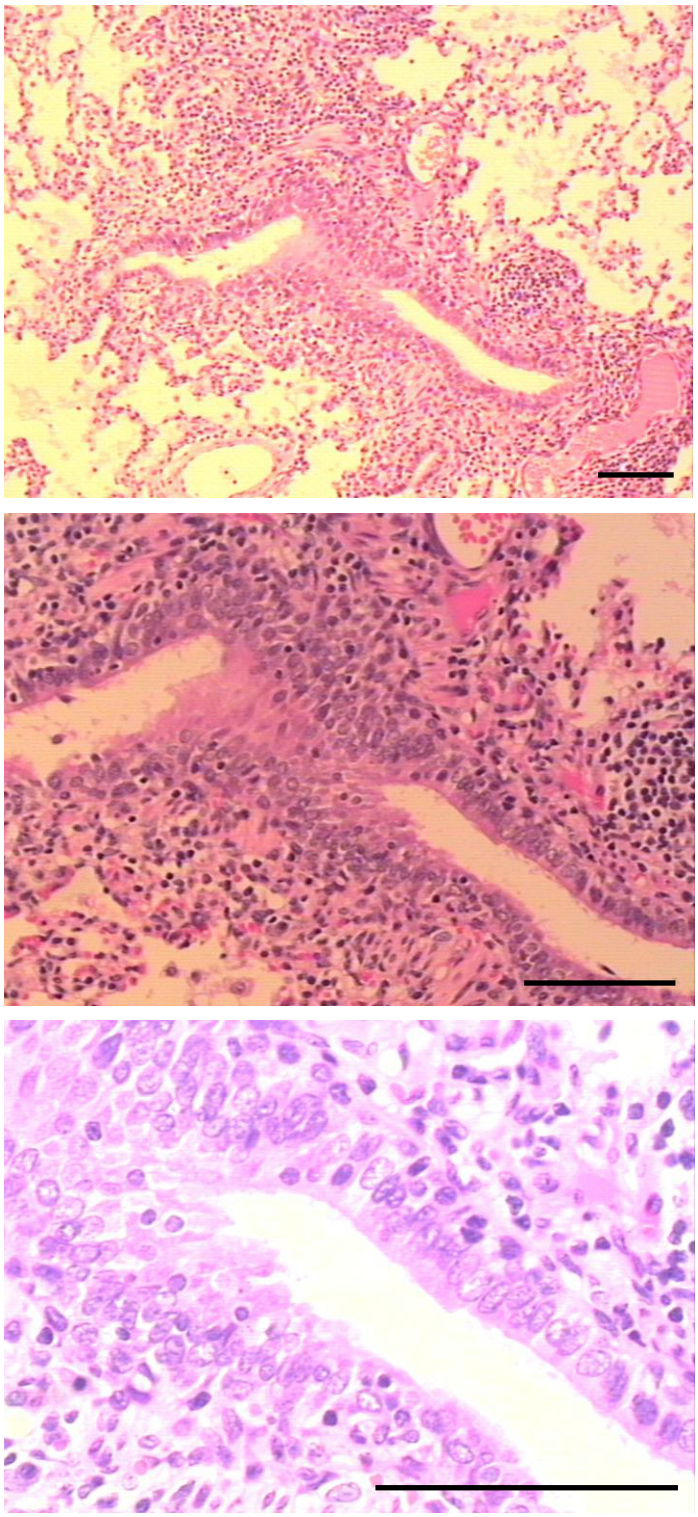


Figure 3.20: Histopathology slides of pig lung exposed to 10 ppm ammonia, at (top to bottom) 10x, 20x and 40x magnification. Scale bar represent 100 μm.

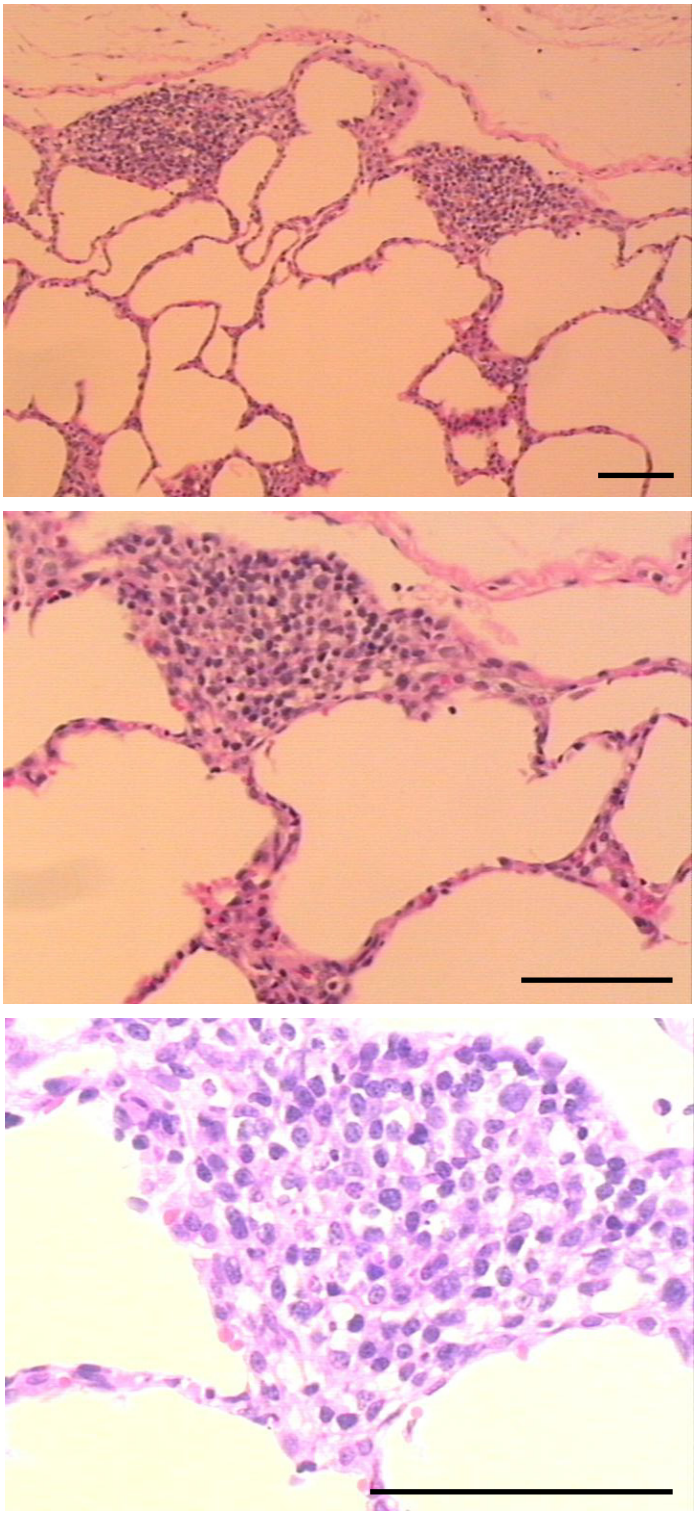


Figure 3.21: Histopathology slides of pig lung exposed to 25 ppm ammonia, at (top to bottom) 10x 40x and 100x magnification. Scale bar represent 100 μm.

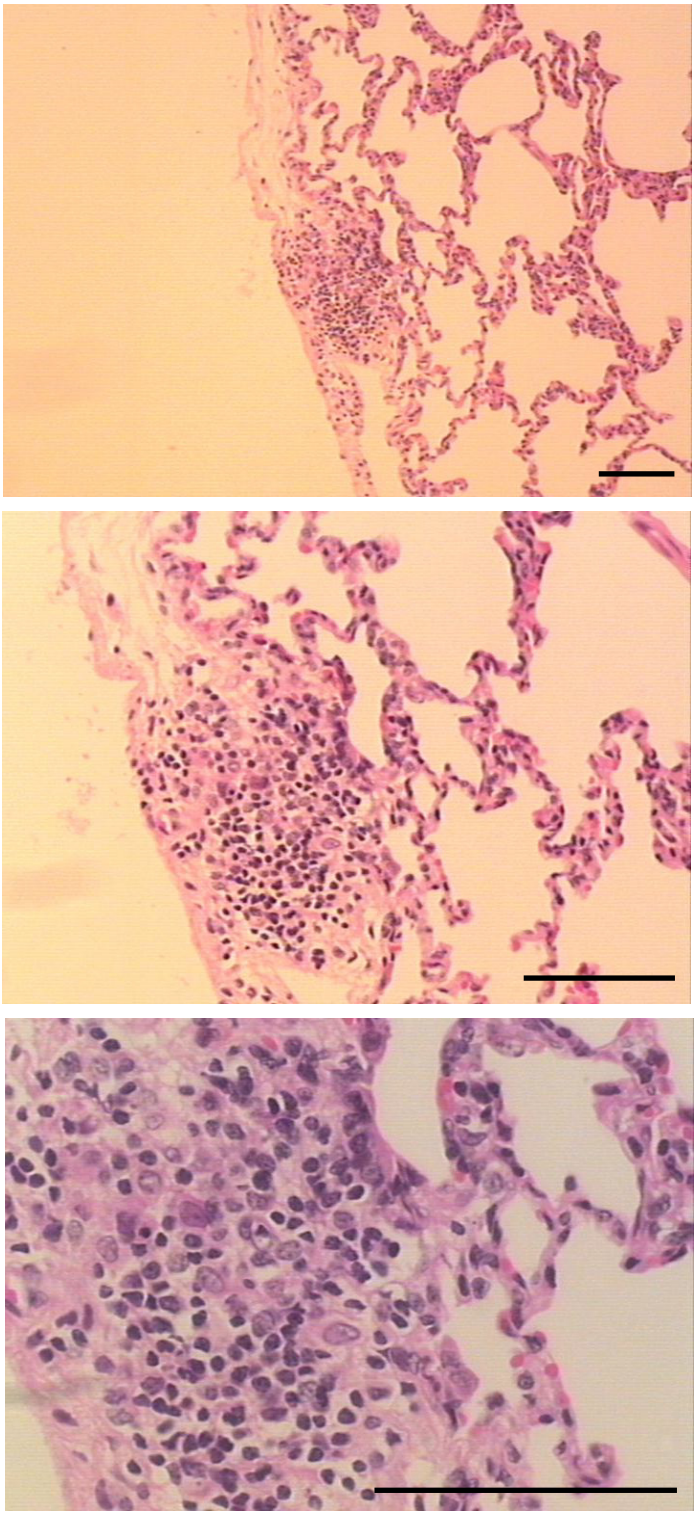


Figure 3.22: Histopathology slides of pig lung exposed to 50 ppm ammonia, at (top to bottom) 10x 20x and 40x magnification. Scale bar represent 100 μm.

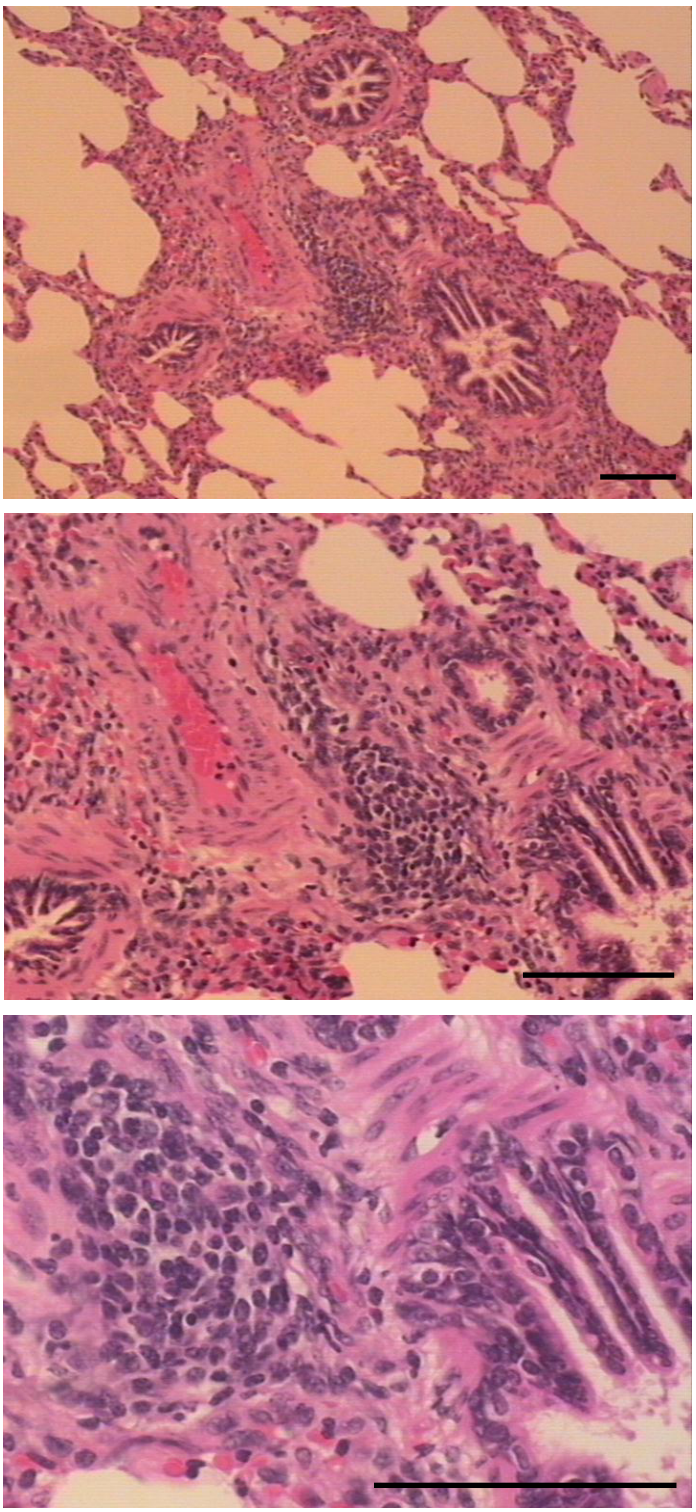


Figure 3.23: Histopathology slides of pig lung exposed to ammonia at 0 ppm and alpha haemolytic cocci (AHC) (200,000 cfu/ml), at (top to bottom) 10x, 20x and 40x magnification. Scale bar represent 100 μm.

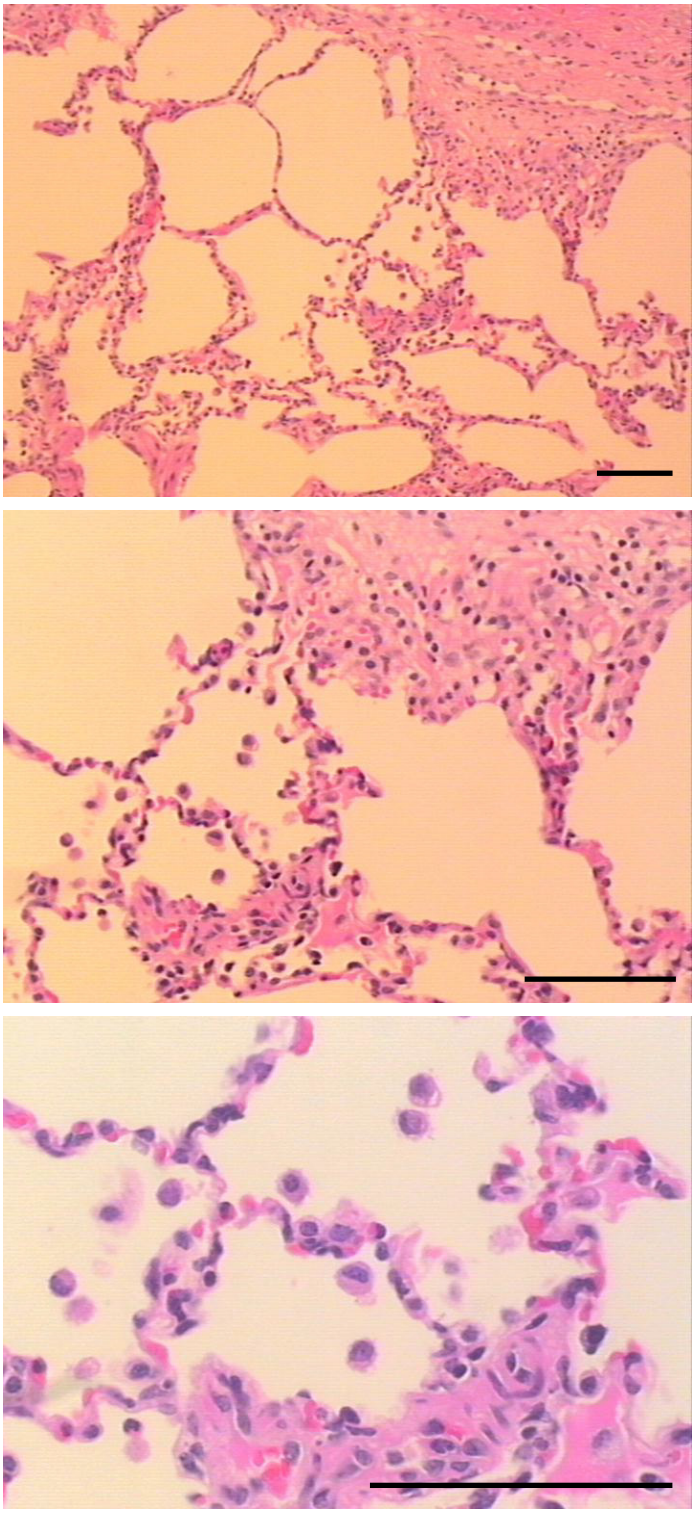


Figure 3.24: Histopathology slides of pig lung exposed to ammonia at a concentration of 10 ppm, and alpha haemolytic cocci (AHC) (200,000 cfu/ml), at (top to bottom) 10x, 20x and 40x magnification. Scale bar represent 100 μm.

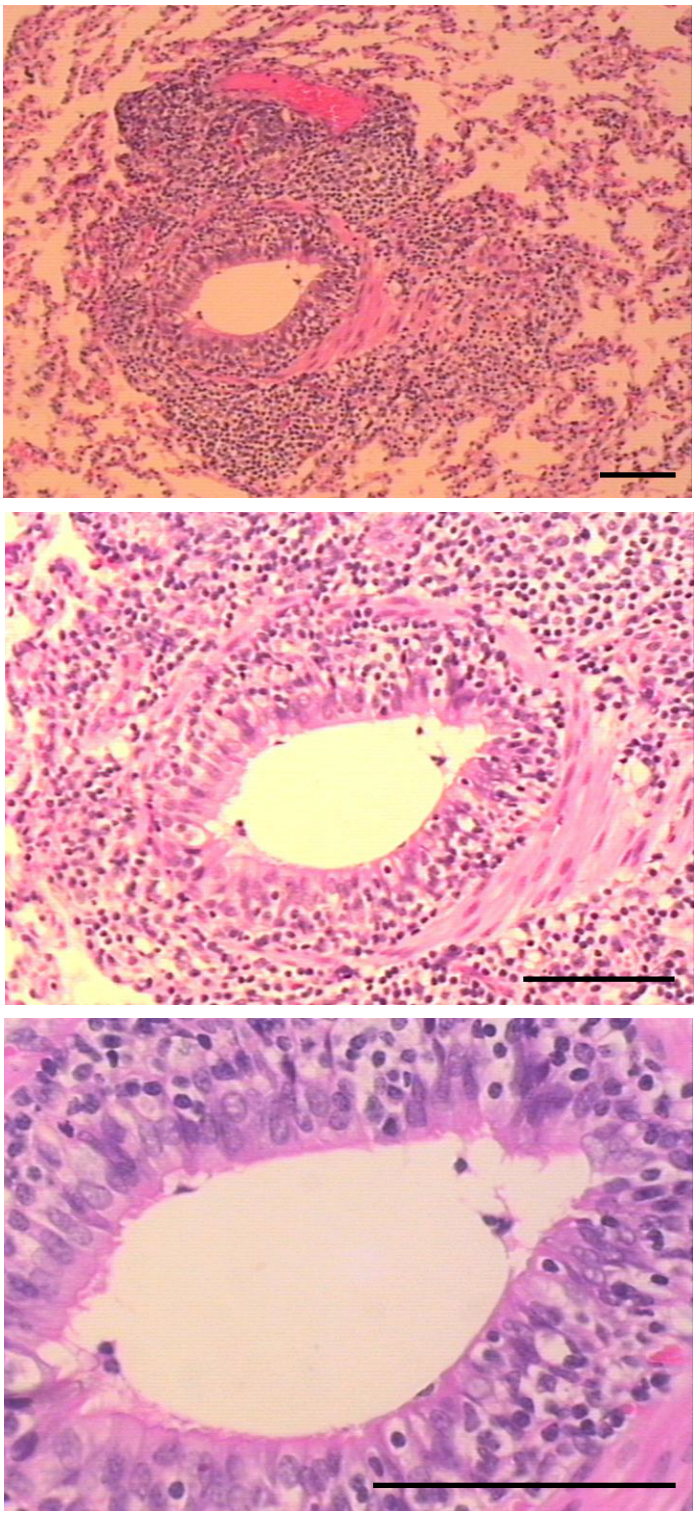


Figure 3.25: Histopathology slides of pig lung exposed to ammonia at a concentration of 25 ppm, and alpha haemolytic cocci (AHC) (200,000 cfu/ml), at (top to bottom) 10x, 20x and 40x magnification. Scale bar represent 100 μm.

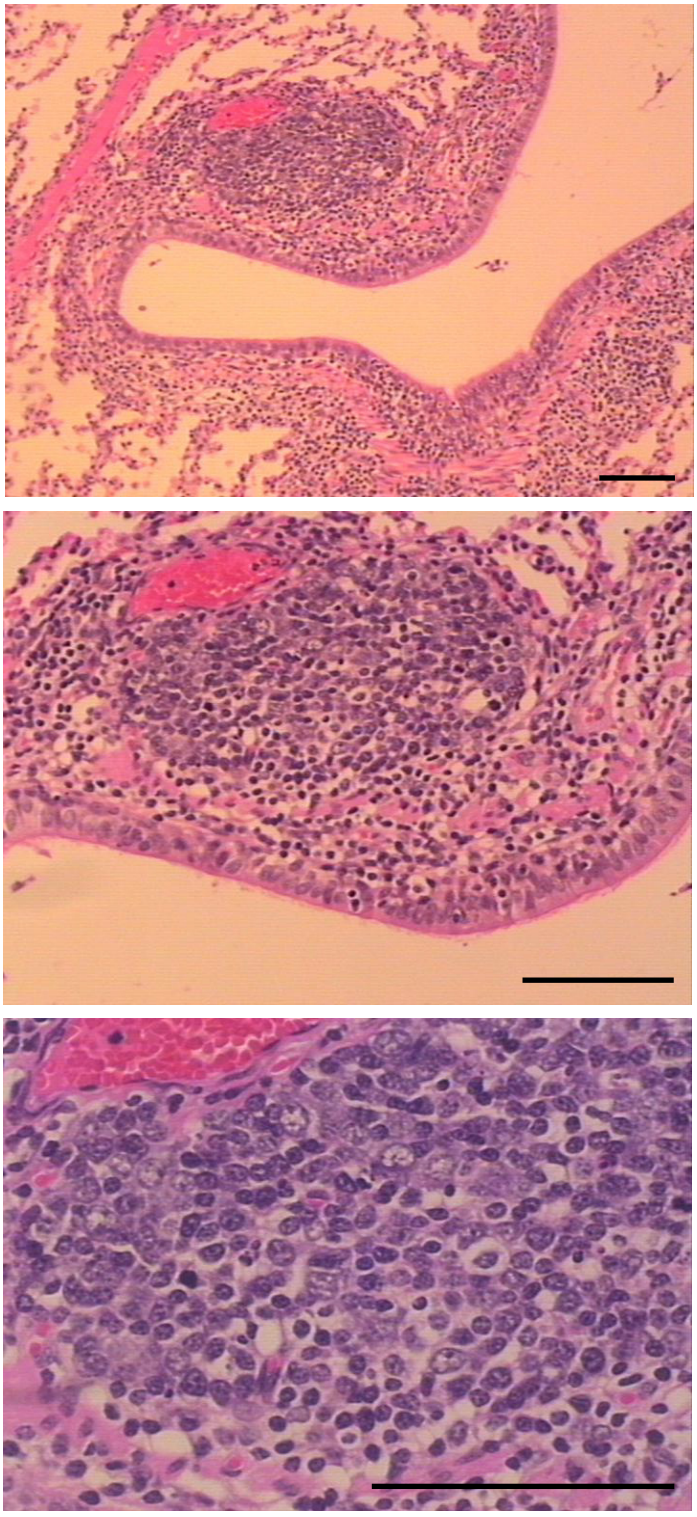
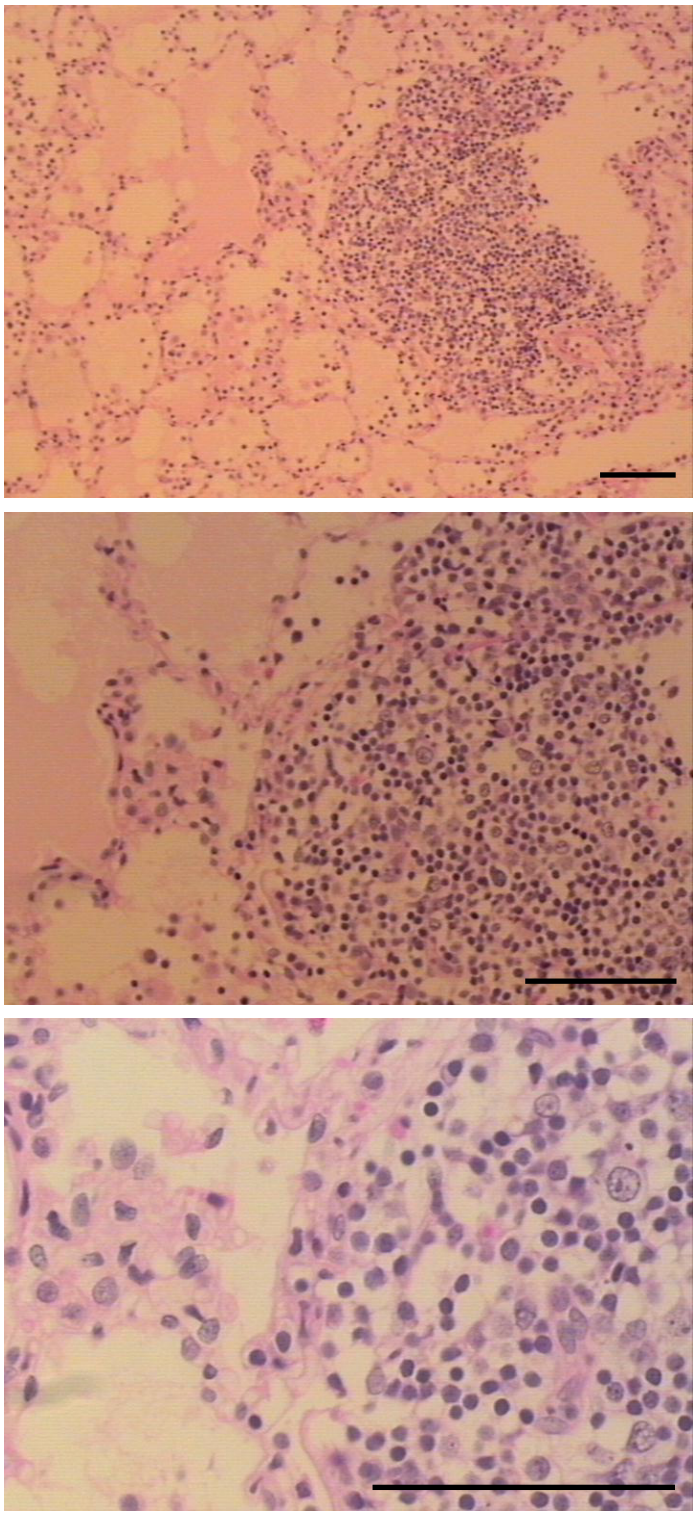


Figure 3.26: Histopathology slides of pig lung exposed to ammonia at a concentration of 50 ppm, and alpha haemolytic cocci (AHC) (200,000 cfu/ml), at (top to bottom) 10x, 20x and 40x magnification. Scale bar represent 100 μm.



3.4 Discussion

The average growth rate and feed conversion ratio in control pigs was 807 g/day and 3.26 respectively, compared to 575 g/day and 3.34, respectively for the Australian pig industry (Australian Pork Limited, 2011).

While exposure to alpha haemolytic cocci (AHC) appeared to have a greater effect than ammonia on growth rate, feed efficiency and feed intake, as well as aspects of immune function, the major effects were observed in pigs exposed to high levels of ammonia followed by (AHC). Skirrow *et al*, (1995) reported that the prevalence of pleurisy was higher in sheds with both high levels of ammonia and bacteria, compared with sheds with concentrations of ammonia below 5 ppm and levels of bacteria above 1.5 $x10^5$ cfu/m^3.

Alpha haemolytic cocci (AHC) were not harmless commensals in the pigs studied. Although there were no visible expressions of disease, there was evidence of decreased growth rate and feed utilisation parameters associated with immune activation.

There is an increasing body of evidence that some species within the AHC group may have pathogenic impacts. In humans, VGS usually act as commensals utilising mucin as an energy substrate (Van der Hoeven *et al*., 1991), but may act as periodontal pathogens (Robertson and Smith, 2009) and may cause rhinosinusitis (Hwang and Tan, 2007). They are also common secondary coloniser in the distal airways of people with chronic lung diseases (Cabello *et al*., 1997). The factors that make the organisms pathogenic are

not known (Hwang and Tan, 2007), but it is known that VGS represent a particular risk to humans with neutropaenia (Tunkel and Sepkowitz, 2002), and that in pigs they readily colonise the aortic valve following mechanical damage to the valve, resulting in endocarditis (Ramirez-Ronda, 1978). *Vagococcus* spp. have been isolated from carcasses of pigs condemned because of pathological changes initially attributed to swine erysipelas, and also from field cases with a presumptive diagnosis of swine erysipelas (Bender *et al*., 2009). *Aerococcus viridans* was first described as a common airborne organism in human-occupied places (Williams *et al*., 1953), and it has also been found in human faecal slurry (Budzinska *et al*., 2009). It is generally considered a saprophyte (Park *et al*., 2005) and very rare as a cause of clinical disease in humans (Popescu *et al*., 2005). However, it has been isolated in pure culture from 11.5% of pigs with arthritis, 2.2% of pigs with meningitis, and 1% of pigs with pneumonia, apparently as an opportunistic pathogen (Martin *et al*., 2007). There is evidence from other host species that some form of immunocompromisation is necessary for the expression of clinical disease (Brauer and Monteil 1983; Dagnæs-Hansen *et al*., 2004). The clinical significances of the Vagococcus spp. have yet to be determined in pigs, but *Vagococcus elongatus* has been isolated from a pig effluent pit (Lawson *et al*., 2007) and *Vagococcus fluvialis* of unknown significance has been isolated from a number of pigs with clinical disease (Pot *et al*., 1994; Teixeira *et al*., 1997).

The conclusion that VGS has sub-clinical impact is not novel; Hanage and Cohen (2002) described a proinflammatory response initiated by VGS in human lung tissues, including upregulation of adhesion molecules and associated neutrophil aggregation in

lungs, and concluded that VGS have the capacity to elicit pathological responses. In my pigs, two of the three measures of growth and feed utilisation (VFI and ADG) were affected adversely to an extent that would be economically important, perhaps because VGS as a group are known to stimulate peripheral blood mononuclear leucocytes to rapidly produce very large amounts of Interleukin (IL)-1β (Hahn *et al*., 2007; Hanage and Cohen, 2002), which could be expected to impact VFI because IL-1β acts directly on the brain to suppress appetite (DeBoer *et al*., 2009). The daily feed loss was 268 g of feed per inoculated pig per day, indicating that 11.0% of the feed was lost to the sub-clinical disease process in those pigs with exposure to AHC, but not ammonia.

The effects of AHC on growth and feed utilisation were associated with activation of the immune system. In this study, across the three measures (change in the marker over 14 day in individual inoculated pigs, difference in the mean levels of markers at the end of the trial between the control and inoculated treatment groups, and changes in the mean levels of the markers during the trial between the control and inoculated treatment groups), lymphocyte stimulation index (LSI), heterophil phagocytic potential (HPP), and CD4, were consistently elevated in pigs inoculated with AHC, but not exposed to ammonia, and CD21, CD8, and the CD4:CD8 ratio were elevated in one measure but not others. These findings are generally consistent with those of other studies that have compared production parameters with the same measures of immune stimulation, but using the unspecified stimulus of unhygienic sheds (LPI and CD4, (Galina-Pantoja *et al*., 2006); CD21, Clapperton *et al*., 2005; 2008); and CD4:CD8 ratio, (Williams *et al*., 1997). However, Clapperton *et al*., (2005) and Galina-Pantoja *et al*., (2006) found

elevated proportions of CD8 markers, whereas I didn't, and Clapperton *et al*., (2005) found no association between elevated CD4 and CD8 and depressed production. Clapperton *et al*., (2005) noted that the specific cell types that change significantly are not necessarily consistent across time or across traits, perhaps varying from pig to pig because of variations in the actual extent of the challenge or because of genetic differences (Gallina-Pantoja *et al*., 2006). This previous research suggests that in the present study the immune system of the pigs was activated by inoculation with AHC, even though not all of the measures of immune response were positive.

The results indicate that hygiene procedures in the present study were not perfect, because there was a moderate increase in the lymphocyte stimulation index and heterophil phagocytic potential in the absence of challenge with either AHC or ammonia. However, the impact of AHC on both parameters was significantly greater than the influence of the occult intercurrent inflammatory agent, and so I have concluded that any impacts by such an agent were minor and unlikely to confound interpretation.

In the present study, acute exposure to ammonia alone may have depressed VFI, but there was no indication of an effect on either ADG or FCR. The most comprehensive previous study of the impact of ammonia on pigs showed no impact of chronic exposure to ammonia over 5.5 weeks, even at the highest concentration tested (37 ppm), on VFI, ADG, or FCR (Wathes *et al*., 2004). Nor was there any association between ammonia and pathological changes in that same study (reported separately by Done *et al*., 2005).

The growth rate and feed utilisation parameters in the studies by Wathes *et al*. (2004) and Done *et al*. (2005) are consistent with the findings of many other studies (Doig and Willoughby, 1971; Curtis *et al*., 1975; von Borell *et al*. 2007), although Diekman *et al*. (1993) found significant depression of ADG at 35 ppm, and Gustin *et al*., (1994) found significant weight loss in pigs exposed continuously to 50 ppm. In the present study, the ammonia was delivered to the feed bin for short periods, rather than being constantly available in the ambient air, but that may have sufficed to suppress VFI.

The lack of a significant impact on FCR suggests that there was an aversion, but no marked activation of the immune system with attendant nutrient demand, in these pigs exposed only to ammonia. Because VFI in pigs may have been affected despite the brief duration of exposure of these pigs to ammonia compared with the constant exposure in the other studies, it was apparent that any aversion would have been due to exposure to ammonia during feeding, and this may be the explanation for the superior growth rates found in pigs held in partially-slatted, compared with fully slatted, pens (Courboulay, 2003), because the feed trough would be closer to the source of the ammonia. However, the evidence is equivocal, because other comparisons of slatted floor types have found no difference (Guingand and Granier, 2001; Rossi *et al*., 2008). Pigs are known to have an aversion to atmospheric ammonia (Jones *et al*., 1996; Smith *et al*., 1996), but I believe that the explanation for the discrepancies between the various studies of growth and feed conversion in pigs exposed to ammonia is likely to be a function of the duration of the study, because Jones *et al*., (1998) found that the lure of food was sufficiently strong to overcome that aversion after 18 days of exposure to ammonia

delivered during feeding. Hence, although I found that exposure to ammonia alone may have adversely impacted VFI, I do not believe that the magnitude of this effect would have economic significance.

The lack of impact of ammonia alone on FCR is consistent with the equivocal impact on immune system parameters evident in my control pigs. I observed slight, but significant, stimulation of heterophil phagocytic activity, an observation consistent with the increases in neutrophils in the nasal cavity observed by Urbain *et al*. (1996a,b) and in the differential white cell count by von Borell *et al*., (2007), and I also observed some CD4 activation, indicating cell-mediated immunity. However, the lack of a proliferative response by lymphocytes and the failure to activate CD21 lymphocytes indicates that there was little, if any, humoral response. Mild inflammatory changes and delayed bacterial clearance have been associated with environmental ammonia by Drummond *et al*., (1978) and Johannsen *et al*., (1987), and Hamilton *et al*., (1998b) detected mild turbinate atrophy due to increased osteoclastic activity in preweaning gnotobiotic piglets, but there is no evidence that ammonia at levels normally prevalent in pig sheds causes major pathological changes or induces major activation of the immune system.

In the present study, ammonia exacerbated the impact of AHC in a substantial, and progressive, manner. An exacerbating effect was also observed in the studies of turbinate atrophy by Hamilton *et al*., (1996, 1998a, 1999), in which the effects on the turbinates of inoculation with *Pasteurella multocida* combined with a continuous supply of ammonia were greater than the individual effects of either agent. However, in that

study the combined effect as measured by a morphometric index was never more than 16% above the effect of the organism alone. In the present study, the combined effect of inoculation with AHC and exposure to 50 ppm ammonia during feeding was to suppress ADG by 39%, and this was associated with elevation of measures of immune function, with the highest percentage increase being of the CD4 marker (34%). A mechanism by which ammonia could facilitate the impact of a micro-organism residing on the nasal mucosa is through its ability to breach the protective mucous and epithelial barriers (Brautbar, 1998), allowing penetration by micro-organism into sub-epithelial tissues. The penetration would largely occur in the upper respiratory tract because the high solubility of ammonia means that it is rapidly dissolved in nasal mucus. The history of VGS as an invader secondary to other agents of epithelial damage (Cabello *et al*., 1997; Hanage and Cohen, 2002; Johnson and Bowie, 1992) suggests that VGS would readily exploit damage caused by ammonia to enable penetration from the mucosal surface into sub-mucosal tissues. Another potential mechanism relates to the affinity of VGS to fibronectin, which selectively promotes the attachment of VGS to oral epithelial cells (Sinner and Tunkel, 2010). Fibronectin is also secreted by endothelial cells, platelets, and fibroblasts in response to vascular injury (Sinner and Tunkel, 2010), and so it may be that ammonia is capable of eliciting a fibronectic response by the vascular tissues that in some way enhances the invasibility of VGS, perhaps by providing the VGS with some protection against phagocytosis. The pathogenesis of *Aerococcus* spp. is much less well understood, but Shannon *et al*. (2010) suggested that aggregation of platelets and fibrin by *Aerococcus urinae* provided protection from antibiotics. A third mechanism might be facilitation of the growth

and/or survival of the organism in the upper respiratory tract of pigs (Hamilton *et al*., 1996), perhaps because of exploitation by the organisms of ammonia as a source of nitrogen, increasing the effective infective dose. Many *Streptococcus* spp. have this capability *in vitro*, e.g. *S. bovis* (Atasoglu and Wallace, 2002); *S. thermophilus* (Monnet *et al*., 2005); and *S. mutans*, (St Martin and Wittenberger, 1980).

The daily feed loss due to the disease process for each pig inoculated with AHC and exposed to 50 ppm ammonia was 667 g, or 34.3% of the feed intake. This is higher than the 15.4% penalty that may be calculated from data presented by Williams *et al*., (1997) for 102 kg pigs from an Iowa herd in which the recognised pathogens *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, swine influenza virus, and transmissible gastroenteritis virus are endemic, but whether the losses in my pigs would have been sustained over an equally long time scale is not known. Clapperton *et al*., (2008), Le Floc'h *et al*., (2004) and Sandberg (2007) postulated that associations between immune traits and performance arose as a result of the nutrient demand of unspecified subclinical disease which diverted energy from growth, and Galina-Pantoja *et al*., (2006) suggested a specific example of such a relationship, between elevated lymphocyte proliferation, low ADG, and subclinical enteric Salmonella infection. Maintaining an immune response is known to have a high energy demand, particularly in species with poor insulation (Hart, 1988), such as the pig; and a severe disease, such as Trypanosomiasis in cattle, may require half again of the maintenance requirements (Akinbamijo *et al*., 1997). Feed losses of these magnitudes are clearly of economic significance. Viridans-group streptococci are early colonisers of the human gut (Park *et*

al., 2005), and are also present in the faeces of pigs in Australia (Skirrow *et al*., 1995). The impact of poor shed hygiene on production parameters and economic performance is well documented (Knowles *et al*., 1997; Le Floc'h *et al*., 2009), and the faecal origins of AHC mean that environmental contamination with AHC would be one consequence of poor shed hygiene. Ammonia is produced from the slurry created by urine and faeces (Groot Koerkamp *et al*., 1998), and so atmospheric ammonia is another consequence of poor shed hygiene (Banhazi *et al*., 2008). I have concluded that AHC is another subclinical disease, prevalent in pig sheds with poor hygiene and exacerbated by the atmospheric ammonia prevalent in those sheds, which activates the pigs' immune system and because of the nutrient demand inherent in immune activation, allows a smaller proportion of the nutrient intake to be directed towards growth.

New Aerococcus and Vagococcus spp. have been recently described, particularly by laboratory groups led by P.A. Lawson (e.g. *Vagococcus elongatus*, Lawson *et al*. 2007). There have also been extensive recent changes in the taxonomy and nomenclature of VGS (Sinner and Tunkel, 2010) and I believe that an investigation aiming to identifying the individual AHC species present in pig faeces and their relative impacts on pig production and on the health of piggery workers is warranted.

This study was unique, in that it was able to demonstrate a successful model to expose individually-housed pigs to individual, or combinations, of airborne pollutants without the need for 'exposure chambers'. In particular, the study was able to determine the effects of ammonia and alpha haemolytic cocci (AHC) on individually-housed pigs.

The results demonstrated that AHC in the absence of ammonia elicited an immune response and depressed growth and feed utilisation parameters, and hence AHC are not commensal. The impacts of AHC are markedly exacerbated by exposure to ammonia, but even though the condition remains sub-clinical, there are impacts on growth and feed utilisation. This study demonstrated that viable AHC contribute to the impact of poor pig shed hygiene on production parameters

4

Effects of stocking density on air quality parameters and growth rate in pigs

4.1 Introduction

Poor air quality is recognised as a major risk factor in the development of respiratory disease in pigs (Donham, 1991). Improved air quality reduces the impact of disease on pig production, resulting in increased growth rate and economic efficiency (Cargill *et al*., 1996). The improved air quality also reduces the occupational health risks associated with pig production (Donham, 1991). Important parameters of air quality include the concentration of respirable particles (RP) and the concentration of bacteria (Bac), especially streptococcal organisms (Skirrow *et al*., 1995).

Stocking density (StD) (m^3 airspace/pig) also has a major effect on air quality. Increasing the stocking density and reducing the number of pigs in the airspace will improve air quality by reducing dust levels and bacterial load within the shed (Cargill *et al.,* 1996). Hence air quality may be improved by dividing large sheds into smaller sections using partitions. The finding that stocking density may reduce air quality in terms of increased bacterial load, and hence, reduce growth rate, in the absence of respiratory disease (Banhazi and Cargill, 1998; Murphy *et al*., 2000), is significant and emphasises the importance of providing adequate airspace for animals. According to current recommendations, pigs weighing 100 kg require 3.0 m^3 airspace/pig (Pointon *et al*., 1995).

As stocking rate (pigs/m^2 floorspace) impacts on pen hygiene, it has also been identified as a risk factor for both enteric (Madec *et al*., 1998; Madec and Leon, 1999) and

respiratory (Skirrow *et al*., 1995) disease. Overcrowding often leads to poor dunging patterns, which reduce standards of hygiene and air quality (Banhazi *et al*., 2000).

It is important to follow guidelines for stocking rates and stocking density in naturally ventilated buildings, where increasing the floor and airspace per pig will have a significant effect on lowering pollutant levels (Skirrow *et al.,* 1995). It is much easier to reduce stocking rate and density than to increase the rate of air exchange.

In this study, the effect of stocking density (StD) on air quality parameters in pig sheds and growth rate was investigated.

4.2 Materials and methods

4.2.1 *Experimental Farms*

A total number of 14 pig farms in South Australia, Victoria and Queensland were used in this study. These farms were active participants in a separate, ongoing research project being conducted by the University of Adelaide and the South Australian Research and Development Institute (SARDI).

4.2.1.1 South Australia and Victorian farms

Eight farms (Farms 2 to 9) were selected for this trial, based on the criterion that each farm had two stage grower units. The first stage consisted of pigs aged 10 to 16 weeks of age and the second stage consisted of pigs aged 16 to 23 weeks of age. All farms

operated with an all-in/all-out (AIAO) production system, all sheds were naturally ventilated and had partially slatted floors. The stocking density of each shed was calculated by multiplying the length by the width and by the average height and divided by the total number of pigs in the shed.

4.2.1.2 Queensland farms

Six farms (Farms 10 to 15) were selected for this trial, based on the criterion that each farm had a series of single stage grower units, or sheds with pigs aged from 10 to 20 weeks. All six farms operated with an all-in/all-out (AIAO) production system, the sheds were naturally ventilated and had partially slatted floors. All pigs on the six farms (Farms 10 to 15) had the same genetic stock and fed the same diets, thus eliminating variability arising from variation in these factors. The stocking density of each shed was calculated by multiplying the length by the width and by the average height and divided by the total number of pigs in the shed.

4.2.2 *Ammonia and carbon dioxide*

Short-term measures of ammonia and carbon dioxide gas concentrations were taken at midday using standard gas tubes (Kitagawa, Komyo Rikagaku Kogyo, Japan). Concentrations were measured at pig breathing level (0.5 metres above slat level) three times at each sample point.

4.2.3 *Airborne particles*

Inhalable and respirable particle concentrations were measured using GilAir pumps (Gilian Instrument Corp., West Caldwell, N.J. USA). These air pumps were connected to cyclone filter heads (for respirable particles) and Seven Hole Sampler (SHS) filter heads (for inhalable particles) (Casella Inc., Kempston, UK) and operated at 1.9 and 2.0 l/min flow rate, respectively. The fibreglass filter papers (Whatman Ltd, USA) were conditioned, following standard operational procedures for gravimetric air sampling (Anon, 1987) by being kept in the laboratory for approximately 24 h before and after deployment. Gillian field calibration instrumentation (Gillian Instrument Corp., West Caldwell, N.J. USA) was used to recalibrate the flow rates of the sampling pumps. The pumps were operated over a 6 or 8 h period which provided a good representation of airborne particles associated with pig activity and feeding times. The selection of the monitoring period was based on a previous study (Pedersen, 1993). After sampling, the filter heads were taken back to the laboratory and the filter paper weighed to the nearest 0.001 milligram using a microbalance (Sartorius MC5, Sartorius AG, Goettingen, Germany) and the respirable and inhalable dust levels were calculated.

4.2.4 *Bacteria*

Total viable airborne bacteria were measured at midday using an Andersen viable six-stage bacterial impactor (Andersen Instruments Incorporated, Atlanta, USA) loaded with Columbia horse blood agar (HBA) plates (Medvet Diagnostics, Adelaide). This time was selected as it is likely to be representative of airborne bacteria associated with pig activity and feeding times. The airspace was sampled for five minutes at a flow rate

of 1.9 l/min. The bacteria plates were incubated for 48 h at 37 ^{O}C and the number of colonies counted manually on top of a light box. The concentration of viable airborne microorganisms was calculated and expressed as colony forming units (cfu/m^3).

4.2.5 *Temperature and humidity*

Temperature and humidity data were recorded using Tinytalk temperature and humidity loggers (Hasting Dataloggers, Tinytalk-1 and 2). Computer software (OTLM) was used to program the loggers pre-deployment and also to download the information. Sensors were used to measure both internal and external temperature and humidity. Sensors were placed as close to pig height level as possible, while still precluding interference by the pigs.

4.2.6 *Feed intake and weight measurement*

Average daily gain (ADG) data was obtained by weighing pigs as they entered sheds at approximately 10 or 17 weeks of age and when they were moved to the next stage or slaughtered at 22-23 weeks. A minimum of groups of 50 pigs (300) was monitored on each farm.

4.2.7 *Data analysis*

Windows based STATISTICA 5.1 (StatSoft Inc., 1996) was used to conduct statistical analysis of the data. Statistical models were developed using analysis of variance (ANOVA) procedures to test treatment effects.

4.3 Results

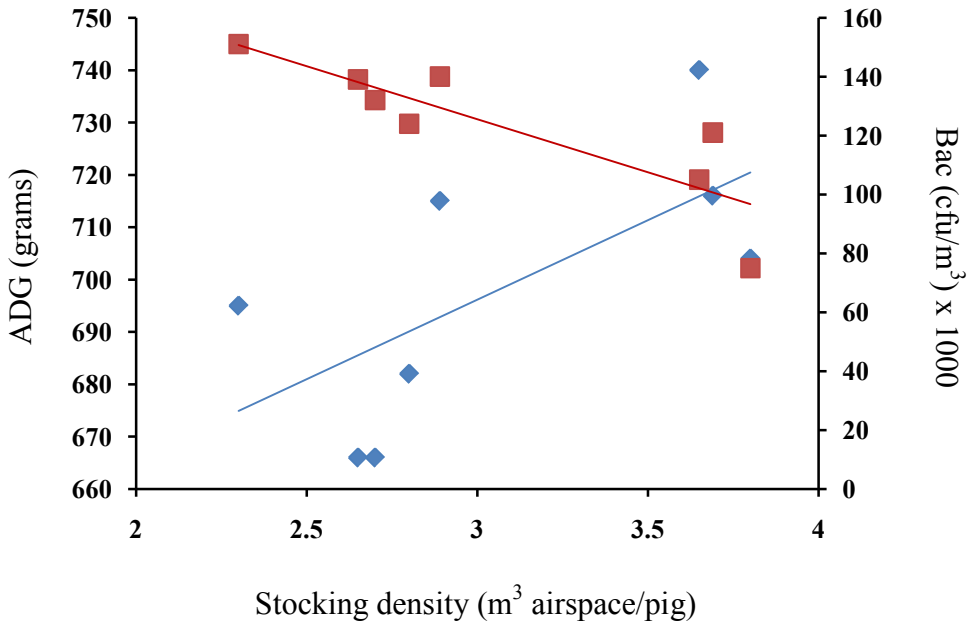
4.3.1 South Australia and Victoria

Stocking density (StD) in stage 1 grow-out units ranged from 2.65 to 3.69 m^3 airspace/pig (Table 4.1) and from 2.70 to 3.80 m^3 airspace/pig in stage 2 grow-out units (Table 4.2).

Table 4.1: The mean growth rate and air quality data in pigs during the stage 1 grow-out period (10-16 weeks) on 8 farms (8 batches/farm). Data are mean values ± SEM.

	ADG g/day	**StD m^3 airspace/pig**	**TD mg/m^3**	**RP mg/m^3**	**Bac cfu x $10^3/m^3$**	**Gram+ cfu x $10^3/m^3$**
Farm 4	666	2.65	2.76	0.27	139	101
Farm 5	704	3.80	1.64	0.21	75	52
Farm 6	682	2.80	1.60	0.19	124	85
Farm 7	715	2.89	1.80	0.24	140	96
Farm 2	695	2.30	2.36	0.28	151	98
Farm 3	666	2.70	1.70	0.23	132	92
Farm 8	716	3.69	0.93	0.17	121	72
Farm 9	740	3.65	2.34	0.18	105	82
	698 ± 9.62	**3.06 ± 0.19**	**1.89 ±0.21**	**0.22 ± 0.01**	**123.4 ± 10.6**	**84.8 ± 8.72**

Figure 4.1: The effect of stocking density on growth rate (◆) and total viable bacteria (■) during the stage 1 grow-out period (10-16 weeks) on 8 farms (8 batches/farm)



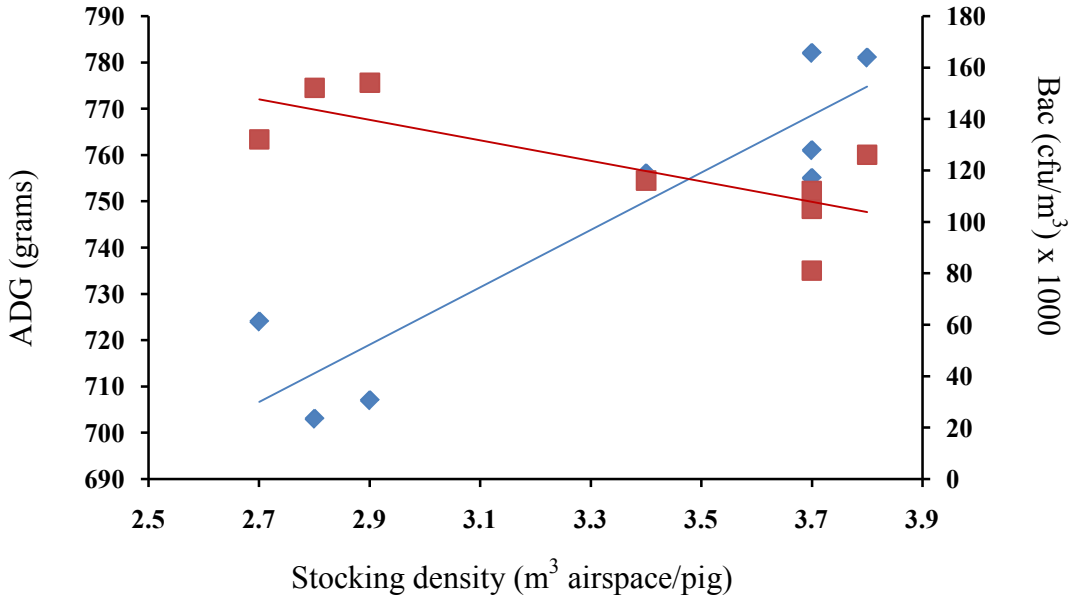
There was a significant negative correlation between StD and Bac (r = -0.85; $P<0.01$), StD and gram positive Bac (r = -0.85; $P<0.01$) and StD and respirable particles (r = -0.78; $P<0.05$) in stage 1 sheds (Figure 4.1). As the volume of airspace provided per pig increased, total dust decreased, but the relationship was not significant at the $P<0.05$ level. By contrast, as the volume of airspace per pig increased, growth rate also tended to increase (r = 0.66; $P=0.07$).

Table 4.2: The mean growth rate and air quality data in pigs during the stage 2 grow-out period (16-23 weeks) on 8 farms (8 batches/farm). Data are mean values ± SEM.

	ADG g/day	**StD m^3 airspace/pig**	**TD mg/m^3**	**RP mg/m^3**	**Bac cfu x $10^3/m^3$**	**Gram+ cfu x $10^3/m^3$**
Farm 2	756	3.42	2.36	0.18	116	60
Farm 3	724	2.70	1.70	0.19	132	108
Farm 8	755	3.69	0.93	0.22	112	87
Farm 9	782	3.65	2.34	0.18	105	88
Farm 4	707	2.92	1.98	0.27	154	125
Farm 5	781	3.80	2.64	0.21	126	95
Farm 6	703	2.80	1.60	0.24	152	112
Farm 7	761	3.69	2.10	0.20	81	46
	755 ± 10.1	**3.44 ± 0.15**	**2.04 ±0.21**	**0.20 ± 0.01**	**118.8 ± 8.92**	**86.4 ± 9.25**

In stage 2 grow-out units (Table 4.2), StD was negatively correlated with Bac (r = -0.74; $P<0.05$) and as the volume of airspace per pig increased there was a trend for respirable particles to decrease. Bac was also negatively correlated (r = -0.75) with growth rate ($P<0.05$), and StD (m^3 airspace/pig) was also positively correlated (r = 0.66) with growth ($P<0.05$) (Figure 4.2).

Figure 4.2: The effect of stocking density on growth rate (◆) and total viable bacteria (■) during the stage 2 grow-out period (16-23 weeks) on 8 farms (8 batches/farm)



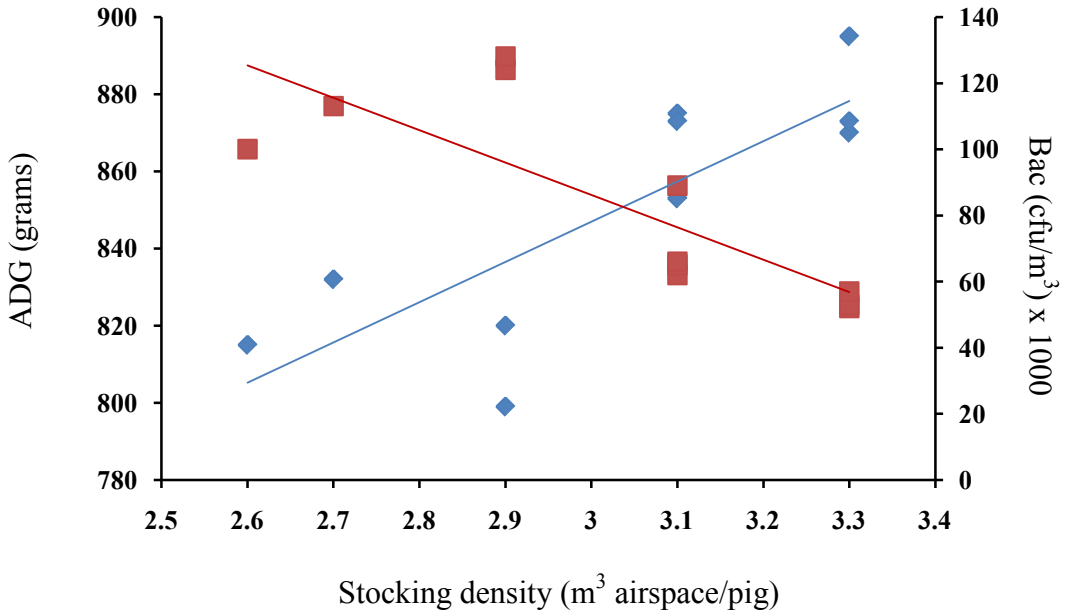
4.3.2 *Queensland*

Stocking density in single stage grower units ranged from 2.65 to 3.26 m^3 airspace/pig (Table 4.3). In the single stage grower units StD (m^3 airspace/pig) was negatively correlated with Bac (r = -0.63; $P<0.01$) but both total dust ($P<0.01$) and respirable particles ($P=0.01$) were positively correlated with StD. There was a strong positive correlation (r = 0.95) between StD (m^3 airspace/pig) and growth rate ($P<0.001$) (Figure 4.3).

Table 4.3: The mean growth rate and air quality data in pigs reared in a single stage grower unit (10-22 weeks) (2 batches/unit). Data are mean values ± SEM.

	ADG g/day	StD m^3 airspace/pig	TD mg/m^3	RP mg/m^3	Bac cfu x $10^3/m^3$
Farm 10	815	2.65	0.32	0.06	99
Farm 11	832	2.73	0.18	0.16	112
Farm 13	873	3.09	0.44	0.30	62
Farm 14	873	3.09	0.36	0.13	64
Farm 12	853	3.10	0.61	0.09	66
Farm 14	895	3.26	1.01	0.40	52
	857 ± 16.6	**2.99 ± 0.14**	**0.49 ± 0.16**	**0.19 ± 0.07**	**76.1 ± 14.6**

Figure 4.3: The effect of stocking density on growth rate (◆) and total viable bacteria (■) during the single stage grower unit (10-22 weeks) (2 batches/unit).



4.4 Discussion

Pointon *et al*., (1995) recommended maximum acceptable levels for various pollutants and husbandary factors as follows: ammonia, 10 ppm; carbon dioxide, 1500 ppm; hydrogen sulphide, 5 ppm; total dust, 2.4 mg/m^3, respirable dust, 0.23 mg/m^3, bacteria 100,000 cfu/m^3, stocking rate, 0.65 m^2/81-100kg pig and stocking density, 3.0 m^3 airspace/100kg pig.

In all of the farms included in this study, there were significant negative correlations between stocking density and bacteria and a significant positive correlation between stocking density and growth rate.

As shown in Table 4.1 (stage 1 growers), all of the farms except one (Farm 5) had higher than recommended levels of bacteria. Farm 5 had the best average stocking density value of 3.80, and below recommended levels of respirable particles and total dust particles.

The farm with the highest recorded bacteria levels (Farm 2) had a very low stocking density value of 2.30 m^3 airspace/pig, well below the recommended level of 3.0 m^3 airspace/pig. The level of respirable dust was slightly above the recommended level (0.28 mg/m^3) and total dust was just under the recommended level at 2.36 mg/m^3. These findings suggest that bacterial load is one of the mediators involved in reduced growth rates associated with overstocking. This would also support the assertion that overstocking the pens and reducing the stocking density leads to a pen with poor hygiene, increased bacteria and concomitant reduced growth rates.

Similar results were observed in stage 2 grow-out units (Table 4.2), with all of the farms, except Farm 7, having higher than recommended levels of bacteria. The three farms with the lowest stocking density had the highest bacteria levels and the lowest values for growth rate. Interestingly, the farm which had the highest value for total dust had the highest growth rate, which may suggest that total dust is not a good indication of pen hygiene. Total dust refers to particles greater than 10 μm and these are usually trapped in the nasal cavity (Gordon, 1963) with only those less than 10 μm proceeding into the trachea. Respirable dust refers to all particles less than 5 μm and can be deposited as deep as the alveoli and air sacs of the lungs (Pedersen *et al*., 2000) where it is most likely that bacteria can be inhaled to initiate an immune response.

The stronger inverse relationship that was observed between respirable particles and average daily gain (ADG) than that between total dust and ADG can also be explained by the fact that respirable particles are generally pig associated, whereas total dust is primarily feed related (Cargill and Skirrow, 1997). The high total dust levels could also be influenced by recent activity, such as staff working in the shed or the feed being delivered.

As shown in Table 4.3, similar results can be observed from the grower units in regards to growth rate, stocking density and bacteria. Farm 15 had the highest average daily gain (895g), the best stocking density (3.26 m^3 airspace/pig) and the lowest recorded bacteria levels (52,052 cfu/m^3). However, respirable dust and total dust levels were well above recommended levels, 0.395 mg/m^3 and 1.01 mg/m^3, respectively. These results suggest

that bacteria and stocking density are the two most important environmental factors for growth rate.

Farm 11 recorded among the lowest total and respirable dust levels, 0.18 mg/m^3 and 0.160 mg/m^3, respectively, yet had the second lowest average daily gain. However, this farm recorded bacteria levels of 112,597 cfu/m^3, higher than the recommended level of 100,000 cfu/m^3.

As previously mentioned, the results from all farms suggest that bacteria and stocking density are the two most important factors that were measured; that is, the ones which have the greatest impact on growth rate. Increasing the volume of airspace per pig appears to have a 'dilution' effect on the bacterial load in the shed. Although some of the units had higher than recommended levels of respirable and total dust, average daily gain did not appear to be compromised. This suggests that many of the particles present in dust do not initiate an immune response which suppresses growth rate.

As the results indicate a close relationship between StD and growth rate and between StD and airborne viable bacteria, it is suggested that StD is a key risk factor for high concentrations of airborne viable bacteria in the airspace, which may in turn compromise the growth rate of pigs. This would also support the hypothesis that overstocking the pens leads to a reduction in hygiene standards, resulting in increased bacteria levels and reduced growth rates.

Based on these results the apparent association between increased stocking density and reduced growth rate is that as the amount of airspace per pig decreases, the standard of surface hygiene is also reduced. This results in an increase in the concentration of bacterial aerosols in the airspace, and based on the data presented in Chapter 3, these aerosols are a major factor in depressing growth rate.

The results confirm the importance of maintaining adequate shed size and limiting the number of pigs housed in naturally ventilated sheds. Controlling these factors will improve air quality and reduce the impact of aerosols on growth rate and respiratory disease in pig herds, as well as reducing occupational health risks for employees (Cargill *et al*., 1996).

Other studies have shown a strong relationship between hygiene and concentrations of airborne bacteria. Skirrow *et al*., (1995) found that the majority of *Streptococcus* spp. recovered in the airspace were of faecal origin and they suggested that the concentration of *Streptococcus* spp. would be a good guide to stocking density and pen hygiene. Stocking rate (pigs/m^2 floorspace) has also been shown to have an impact on pen hygiene and has been identified as a risk factor for both enteric (Madec and Leon, 1999) and respiratory (Skirrow *et al*., 1995) disease. Overcrowding is also associated with poor dunging patterns, which in turn reduces hygiene standards (Banhazi *et al*., 2000). The finding that stocking density may reduce air quality in terms of increased bacterial load, and hence reduce growth rate in the absence of respiratory disease (Banhazi and Cargill, 1998, Banhazi and Cargill, 1999) is significant and emphasises the importance

of providing adequate airspace for pigs. According to current recommendations, pigs weighing 100 kg require 3.0 m^3 airspace/pig (Pointon *et al*., 1995). The data from the present study supports this recommended value.

Cormier *et al*., (1990) measured airborne micro-organisms in two types of pig buildings (farrowing and fattening units) and found that the predominant micro-organisms were gram positive bacteria, with small quantities of gram negative bacteria, yeasts and moulds. Identification of the colonies revealed a great diversity of micro-organisms. Although there were some slight differences in airborne microbial flora in farrowing and fattening units, the level of airborne microbial contamination did not vary significantly as a function of the outside temperature. However, in other studies (Butera *et al*., 1991), temperature appeared to influence the concentration of viable bacteria and the concentration of organisms was less at higher temperatures. The effect of humidity was more variable. Of potentially greater importance was the fact that some species of bacteria and fungi isolated are known to induce extrinsic allergic alveolitis (Cormier *et al*., 1990) and other fungi are known to be potentially pathogenic for humans.

There is a strong correlation between stocking density in terms of m^3 airspace/pig and airborne bacteria counts (Wathes, 1994; Murphy *et al*., 2000). Both the concentration of viable bacteria in the airspace, as well as stocking density (m^3 airspace/pig) have been shown to be negatively associated with growth rate (Murphy *et al*., 2000). This finding suggests that bacterial load is one of the mediators involved in reduced growth rates associated with overstocking.

5

Effects of improving shed design and management on air quality parameters and growth rate in pigs

5.1 Validation of strategies for reducing selected air pollutants – 4 case studies

5.2 Introduction

The standard of surface and air hygiene within animal houses is dependent on a series of complex interactions between building design and animal management and behaviour. Shed design factors include the shape and dimensions of the building, the type of system used for ventilation, thermal control, effluent management and the type and quality of the bedding. Animal management factors include the type of production system, as well as the stocking density, stocking rate and the age of the animals. Behavioural traits, such as dunging patterns, animal activity, aggression and social interaction can also influence hygiene and air quality (Cargill *et al.,* 1997; Cargill and Banhazi, 2002).

Significant positive health and production benefits, as well as improvements in shed hygiene and air quality, and a marked reduction in the use of antibiotics, have been achieved by converting herds to a batch-farrowing/age-segregated rearing (BF-ASR) production system (Cargill *et al*., 1998). Research in Australia, Europe and North America has confirmed the value of adopting more innovative management systems to improve air and surface hygiene in both new and existing sheds (Crowe *et al*., 1994; Cargill *et al*., 1996; Cargill *et al*., 1997; Banhazi and Cargill, 1998; Cargill *et al*., 1998; Banhazi *et al*., 1999; Madec and Leon, 1999; Cargill *et al*., 2000). Husbandry systems, such as batch farrowing, segregated early weaning, age-segregated rearing, and multi-

site production, all of which incorporate all-in/all-out (AIAO) with cleaning between batches, enable higher standards of hygiene and air quality to be achieved.

Because effluent is a major source of a number of key airborne pollutants, factors such as the type of effluent system, the use of recycled water, and the distance between the surface of the slurry and the base of the slats (Madec and Leon, 1999) all impact on air and surface hygiene. Broken and blocked slats, as well as air entering the shed through openings over the pits at the end of the sheds, will exacerbate the problem. Modifying diets by lowering protein levels and improving amino acid balance, and adding yucca extracts and enzymes (Cole, 1994) has also be shown to reduce ammonia emissions. There are also a variety of aerobic and anaerobic digestion systems, as well as slurry activators, which can be used to reduce the species and amounts of emitted air contaminant of slurry, including ammonia, hydrogen sulphide, and methane.

Ensuring that effluent disposal systems operate effectively is important to air quality inside pig buildings. One of the best solutions is to use slatted floors over effluent channels and to remove dung frequently, using a scraper, followed by flushing. In the absence of scraping, an alternative manure removal system is to flush effluent channels frequently with a large quantity of fresh water (Groenestein, 1994). Emptying the pits less frequently has also been shown to reduce ammonia emissions (Cargill and Skirrow, 1997). In Australia, the use of recycled water for economic reasons creates air quality problems in a country where the use of fresh water would add a significant cost factor to the operation (Cargill and Banhazi, 2002).

Another factor to consider is the dunging pattern in the shed as this will have a great influence on pen hygiene and hence air quality. Although the causes of poor dunging patterns have not been clearly defined, overcrowding, draughts, and wet floors are known to be significant factors (Banhazi *et al*., 2000). Practices that may encourage good dunging patterns include ensuring floors are dry before restocking, eliminating draughts by covering gaps between walls and shutters and keeping doors closed. It is also important to adhere to the recommended stocking levels (Cargill and Banhazi, 2002).

5.3 *Experimental Farms*

Four farms in South Australia, Victoria and Queensland (Farms 16 to 19), representative of pig buildings in Australia, were used in these case studies. These farms were part of a broader study by the South Australian Research and Development Institute (SARDI), had been identified as having structural and/or management problems, and were willing to incorporate these case studies into their schedule.

5.4 Case study one – the effect of renovation and stocking density on air quality parameters and growth rate

5.4.1 *The farm*

The shed assessed in this study (Farm 16) was a grower-finisher, naturally ventilated, partially slatted shed that was part of an age-segregated rearing (ASR) all-in/all-out (AIAO) production system. The case study involved an assessment of the shed to

identify deficiencies known to reduce air quality and growth rate prior to renovation to remove the deficiencies.

During April 2000, an assessment of the facility using the Hygiene Air Quality (HAQ) index (an in-house evaluation tool assessing sheds to predict hygiene and air quality) identified deficiencies with the ridge vent and overstocking. The ridge vent was narrow (400 mm) and had a low ridge cap (250 mm between the roof and the gap). The stocking density was 2.7 m^3 airspace/pig (recommended target level is 3.0 m^3 airspace/pig).

In February 2001, the shed was renovated by widening the ridge vent from 400 mm to 1000 mm and raising the ridge cap to 400 mm above the opening. Blinds were attached to the sides of the ridge vent openings. The stocking density was increased to 3.0 m^3 airspace/pig.

5.4.2 *Materials and methods*

The initial assessment of air quality parameters, prior to the modifications, was undertaken in April/May 2000. The modifications to the shed followed in February 2001, and the impact of those modifications on air quality were assessed in May 2001.

Two groups of pigs were monitored in 2000 prior to the renovation. The pigs were of the same genetic stock and fed the same diets. The growth rates for the first group of pigs were assessed for the period from 1st March to 17th May (Autumn). The growth rates for the second group of pigs were assessed for the period from 26th May to 11th

August (Winter). A further two groups of pigs were monitored in 2001 after the renovations. The growth rates for the first group of pigs were assessed for the period from 5th March to 24th May (Autumn). The growth rates for the second group of pigs were assessed for the period from 28th May to 15th August (Winter). This was done to minimise any potential seasonal effects on growth rate and air quality. Pigs were housed in the shed from approximately 10 weeks of age until slaughter at approximately 21 weeks of age. The shed was managed as an AIAO production system. The air quality parameters measured were airborne respirable and inhalable particles (mg/m^3), total number of airborne viable bacteria (cfu/m^3), ammonia gas (ppm), and carbon dioxide gas (ppm). The air quality parameters were measured during weeks 3 and 7 of the trial and results were pooled. The side curtains were opened half way during the sampling period to avoid variation in the ventilation rate.

5.4.2.1 Ammonia and carbon dioxide

Short-term measures of ammonia and carbon dioxide gas concentrations were taken at midday using standard gas tubes (Kitagawa, Komyo Rikagaku Kogyo, Japan). Concentrations were measured at pig breathing level (0.5 metres above slat level) three times at each sample point.

5.4.2.2 Airborne particles

Inhalable and respirable particle concentrations were measured using GilAir pumps (Gilian Instrument Corp., West Caldwell, N.J. USA). These air pumps were connected to cyclone filter heads (for respirable particles) and Seven Hole Sampler (SHS) filter

heads (for inhalable particles) (Casella Inc., Kempston, UK) and operated at 1.9 and 2.0 l/min flow rate, respectively. The fibreglass filter papers (Whatman Ltd, USA) were conditioned, following standard operational procedures for gravimetric air sampling (Anon, 1987) by being kept in the laboratory for approximately 24 h before and after deployment. A field blank (matched-weight filter cassesette using filter papers from the same batch used for sampling, with no air drawn through it) was used at each sampling site. Gillian field calibration instrumentation (Gillian Instrument Corp., West Caldwell, N.J. USA) was used to recalibrate the flow rates of the sampling pumps. The pumps were operated over an 8 h period which provided a good representation of airborne particles associated with pig activity and feeding times. The selection of the monitoring period was based on a previous study (Pedersen, 1993). After sampling, the filter heads were taken back to the laboratory and the filter paper weighed to the nearest 0.001 milligram using a microbalance (Sartorius MC5, Sartorius AG, Goettingen, Germany) and respirable and inhalable dust levels were calculated.

5.4.2.3 Bacteria

Total viable airborne bacteria were measured at midday using an Andersen viable six-stage bacterial impactor (Andersen Instruments Incorporated, Atlanta, USA) loaded with Columbia horse blood agar (HBA) plates (Medvet Diagnostics, Adelaide). This time was chosen as a good representation of airborne bacteria associated with pig activity and feeding times. The airspace was sampled for five minutes at a flow rate of 1.9 l/min. The bacteria plates were incubated for 48 h at 37 ^{O}C and the number of colonies were counted manually on top of a light box. The concentration of viable

airborne microorganisms were calculated and expressed as colony forming units (cfu/m^3).

5.4.6 *Data analysis*

Windows based SPSS 17.0, (SPSS Inc, Chicago, USA, 2009) was used to conduct statistical manipulation of the data. Statistical models were developed using analysis of variance (ANOVA) procedures to test treatment effects.

5.4.7 *Results*

Table 5.1: The average growth rate and air quality data for pigs housed in sheds before and after renovations (March – May) (Autumn). Data are mean values ± SEM.

Year	2000	2001
Growth rate (g/day)	750 ± 5.6^a	780 ± 6.0^b
Ammonia (ppm)	11.0 ± 0.46^a	5.0 ± 0.28^b
Viable airborne bacteria (cfu's x $10^3/m^3$)	133 ± 2.60^a	105 ± 2.16^b
Respirable particles (mg/m^3)	0.255 ± 0.004^a	0.194 ± 0.002^b
Inhalable particles (mg/m^3)	2.41 ± 0.03^a	2.16 ± 0.02^b

Differing superscripts within a row indicate a significant difference ($P<0.05$)

The recommended maximum levels for air quality parameters are ammonia (10ppm), viable airborne bacteria (100,000 cfu/m^3), respirable particles (0.23 mg/m^3) and inhalable particles (2.4 mg/m^3) (Donham, 1995; Banhazi *et al*., 2008).

All air quality parameters were reduced (P<0.01) in 2001, after the renovation, compared with 2000 (Table 5.1). Prior to the modifications respirable dust, inhalable dust, viable airborne bacteria and ammonia were above the recommended maximum levels. All parameters, except viable airborne bacteria, were reduced below the recommended limit after the modifications.

During the trial period (77 days), the average daily gain after the shed renovation increased from 750 g in 2000 to 780 g in 2001. This equates to an increase of 30 g/day or 2.3 kg over 77 days.

The improved average gain can be attributed to improved air quality from improving stocking density from 2.7 m^3 airspace/pig to 3.0 m^3 airspace/pig and improved shed design. No other changes in management were identified that could explain the improvements in growth rate and air quality parameters.

Table 5.2: The average growth rate and air quality data for pigs housed in sheds before and after renovations (May – August) (Winter). Data are mean values ± SEM.

Year	**2000**	**2001**
Growth rate (g/day)	753 ± 4.9^a	785 ± 5.5^b
Ammonia (ppm)	10.3 ± 0.41^a	5.0 ± 0.27^b
Viable airborne bacteria (cfu's x $10^3/m^3$)	134 ± 2.76^a	107.5 ± 2.39^b
Respirable particles (mg/m^3)	0.245 ± 0.005^a	0.192 ± 0.003^b
Inhalable particles (mg/m^3)	2.36 ± 0.040^a	2.25 ± 0.013^b

Differing superscripts within a row indicate a significant difference (P<0.05)

All air quality parameters were improved ($P<0.01$) in 2001, after the renovation, compared with 2000 (Table 5.2). Prior to the modifications respirable dust, viable airborne bacteria and ammonia were above the recommended maximum levels. All parameters, except viable airborne bacteria, were reduced to below the recommended limit after the modifications.

During the trial period (77 days), average daily gain after the shed renovation increased from 753 g in 2000 to 785 g in 2001, after the shed renovation. This equates to an increase of 32 g/day or 2.4 kg over 77 days.

5.4.8 *Discussion*

The results indicate a close relationship between growth rate, stocking density, ammonia, bacteria and dust particles. During the trial the stocking density was increased from 2.7 m^3 to 3.0m^3 of available airspace per pig. Stocking density has been shown to have a major influence on air quality and pen hygiene, as well as being a risk factor for enteric disease (Madec and Leon, 1999) and respiratory disease (Skirrow *et al*., 1995). Overcrowding is also associated with poor dunging patterns, which in turn reduce hygiene and air quality standards (Banhazi *et al*., 2000). The improvements observed between stocking density and air quality parameters are in accordance with previous studies (Cargill *et al*., 1996; Murphy and Cargill, 2004). They demonstrated that increasing stocking density will improve air quality by reducing dust levels and bacterial load within sheds.

Both stocking density (m^3 airspace/pig) and stocking rate (pigs/m^2 floorspace) have a major influence on air quality and pen hygiene and both have been identified as risk factors for enteric (Madec and Leon, 1999) and respiratory (Skirrow *et al*., 1995) disease. Overcrowding is also associated with poor dunging patterns, which in turn reduce hygiene and air quality standards (Banhazi *et al*., 2000). Increasing stocking density will improve air quality by reducing dust levels and bacterial load within sheds (Cargill *et al*., 1996). The finding that stocking density may reduce air quality in terms of increased bacterial load, and hence reduce growth rate in the absence of respiratory disease (Murphy *et al*., 2000; Banhazi and Cargill, 1998), is significant and emphasises the importance of providing adequate airspace for animals.

In terms of air quality, ammonia is the most common gas present in pig sheds that affects the health and welfare of both pigs and humans (Payne, 1994; Cargill and Skirrow, 1997). The main source of ammonia is the slurry of dung and urine. After the renovations, the ammonia level in the sheds decreased to below the recommended level of 10 ppm. The reduction in ammonia levels is most likely due to improvements in ventilation and hygiene due to increasing the stocking density. A study by Banhazi *et al*., (2000) demonstrated that reducing the ventilation to maintain an optimal thermal environment increased the concentrations of pollutants and reduced air quality. Cargill and Banhazi (2002) demonstrated that increasing ventilation by purging, or flushing the airspace, or opening shutters for short periods cleared both carbon dioxide and ammonia levels, without a long-term drop in temperature. Increasing the ventilation rate from 20 to 60% reduced ammonia levels in pig sheds from 16 ppm to 7 ppm (Kim *et al*., 2007).

Viable bacteria levels were reduced to just above acceptable recommended levels. There is a strong correlation between stocking density and airborne viable bacteria (Wathes, 1994) which has been shown to be negatively associated with growth rate (Murphy *et al*., 2000).

The improvements in shed design was associated with a substantial reduction in the respirable particles to below recommended levels. There was also a slight reduction in inhalable dust particles. Pedersen (1989) demonstrated that low humidity, as well as very high and low levels of ventilation, resulted in increased airborne dust levels. The major source of dust is feed, but as most of these particles range from 10 μm to 100 μm; feed has little effect on the concentration of respirable dust (Cargill and Skirrow, 1997). Many respirable dust particles contain enteric bacteria and endotoxins, suggesting that they originate from dung (Pickrell *et al*., 1993). The number of pigs in the airspace, as well as pig and human activity, are key factors influencing the concentration of dust found in sheds (Gustafsson, 1994; Skirrow *et al*., 1995; Cargill *et al.,* 1996).

The key housing factors that are likely to influence air and surface hygiene include shed height, angle of the roof pitch, and the space available for sidewall openings, all of which determine the amount of airspace provided, and influence ventilation rate in naturally ventilated buildings (Cargill and Skirrow, 1997). Other factors include the width of the ridge vent, and the height of the ridge cap. Cargill *et al.,* (2000) have recommended that sidewall openings should be a minimum of 20% of the width of the shed and the width of the ridge vent at least 10% of the width of the shed. The angle for

the roof pitch should be a minimum of 15 degrees. Prior to the renovations, the ridge vent and height of the ridge cap were below the recommended levels by Cargill *et al*., (2000). As part of the renovation, the ridge vent was widened from 400 mm to 1000 mm and the ridge cap was raised to 400 mm above the opening. The sidewall openings and roof pitch were above the recommended levels prior to and after the renovations.

Ventilation is a key factor in reduced air quality and in a majority of buildings ventilation rates are designed to optimise air temperature. However, in most situations this results in a build-up of airborne pollutants (Banhazi *et al.,* 2000). In general, as ventilation rate increases, the level of air pollutants decreases and air quality improves (Nicks *et al.,* 1989). However, this only applies when a high standard of surface hygiene is maintained and stocking rates are optimal. In sheds with dirty floors, increasing ventilation rates will reduce air quality and it has also been demonstrated that ventilation rates cannot compensate for sub-standard hygiene (Banhazi *et al.,* 2000).

In this experiment, the stocking density was increased from 2.7 m^3 airspace/pig to 3.0 m^3 airspace/pig after the renovation. As seen in Chapter 5, increasing the stocking density improved air quality by reducing the levels of ammonia, respirable dust, inspirable dust and airborne viable bacteria. The improvement in air quality were associated with an improvement in growth rate. A study by Cargill *et al*., (1996) demonstrated that increasing stocking density led to an improvement in air quality by reducing dust levels and bacterial load within the shed.

In the present study, the ventilation was improved by renovating the ridge vent. The increase in stocking density led to an improvement in animal and surface hygiene, and the improvement in hygiene after the renovation was associated with lower levels of airborne pollutants and an improvement in air quality. As no other changes were made to management, housing, environment, nutrition or genetics, the improvement in growth rate can be attributed to changes to stocking density and shed ventilation. Improved growth rates were achieved after renovations presumably as a result of the improvement in ventilation and stocking density, which allowed more airspace per pig. This 'dilution effect' improved the air quality by reducing concentrations of respirable dust, inspirable dust, viable bacteria and ammonia.

5.5 Case study two – the effect of re-stocking time on pen hygiene, air quality parameters and growth rate

5.5.1 *The farm*

The shed assessed on this farm (Farm 17) was a grower-finisher naturally ventilated shed operated with an all-in/all-out (AIAO) production system. The purpose of this study was to assess the effect of wet versus dry floors prior to re-stocking.

The grower-finisher shed had been previously divided into two sections (A and B) of equal size with 25 pens/section. Each section was de-stocked and re-stocked at the same time and pens were fitted with partially slatted floors, with slats taking up approximately 41% of the floor area.

5.5.2 *Materials and methods*

Following de-stocking, the sections were cleaned and disinfected and left to dry. Section A was re-stocked within 24 hours of cleaning and it was noted that 13 of the pens were still partially wet. Section B was re-stocked three days later when all pens were dry. The stocking rate for both sections was 0.75 m^2 floorspace/pig with 12 pigs per pen and the stocking density was 2.80 m^3 airspace/pig.

Six and eight weeks after the pens were stocked, the shed was assessed using the Hygiene Air Quality (HAQ) index, and hygiene and air quality monitored. The air quality parameters measured were respirable dust, bacteria and ammonia gas. The side curtains were opened half way during the sampling period to avoid variation in the ventilation rate.

Sixty pigs in each section were identified and weighed into the shed and again 8 weeks later at pre-sale to determine average daily gain (ADG).

5.5.2.1 Ammonia and carbon dioxide

Short-term measures of ammonia and carbon dioxide gas concentrations were taken at midday, as described in 5.4.2.1.

5.5.2.2 Airborne particles

Inhalable and respirable particle concentrations were measured, as described in 5.4.2.2.

5.5.2.3 Bacteria

Total viable airborne bacteria were measured, as described in 5.4.2.3.

5.5.3 *Data analysis*

Windows based STATISTICA 5.1 (StatSoft Inc, 1996), SPSS 17.0, (SPSS Inc, Chicago, USA, 2009), Statistix 8, and Excel were used to conduct statistical manipulation of the data. Statistical models were developed using two-way repeated ANOVAs to test time and section effects. The experiment was pseudo-replicated.

5.5.4 *Results*

Table 5.3: Pen condition and air quality parameters, 6 weeks after restocking pens left wet (section A) and dry (section B). Data are mean values ± SEM.

Parameter	Section A	Section B
% floor wet	48.8 ± 21.6^{a}	20.0 ± 10.8^{b}
% dunged floor	40.8 ± 18.9^{a}	14.8 ± 9.18^{b}
Ammonia (ppm)	8.3 ± 2.6^{a}	3.6 ± 1.2^{b}
Viable airborne bacteria (cfu's x $10^3/m^3$)	142.5 ± 8.89^{a}	101.3 ± 7.7^{b}
Respirable particles (mg/m^3)	0.242 ± 0.01^{a}	0.223 ± 0.01^{b}

Differing superscripts within a row indicate a significant difference ($P<0.05$)

5.5.4.1 Six weeks post stocking

In section A (restocked within 24 hours), 48.8% of the pens were wet (water/urine), compared to 20.0% for section B (restocked after 3 days). In section A, 40.8% of the pens had manure on the pen floor, compared to 14.8% for section B (Table 5.3).

All air quality parameters were reduced (P<0.01) in Section B (restocked after 3 days) compared to Section A (restocked within 24 h). The concentration of ammonia was reduced (P<0.01) in section B compared to section A, with 3.6 ppm and 8.3 ppm respectively. Both of these values were under the maximum acceptable limit of 10 ppm. All air quality parameters were reduced to below maximum acceptable levels except viable airborne bacteria.

5.5.4.2 Eight weeks post stocking

Table 5.4: Average growth rate, pen condition and air quality parameters, 8 weeks after restocking pens left wet (section A) and dry (section B). Data are mean values ± SEM.

Parameter	Section A	Section B
Growth rate (g/day)	612 ± 4.0^{a}	643 ± 3.0^{b}
% floor wet	53.60 ± 3.91^{a}	23.20 ± 2.14^{b}
% dunged floor	46.4 ± 3.55^{a}	20.4 ± 1.78^{b}
Ammonia (ppm)	10.3 ± 1.33^{a}	4.3 ± 0.33^{b}
Viable airborne bacteria (cfu's x $10^3/m^3$)	152.5 ± 5.64^{a}	106.0 ± 3.50^{b}
Respirable particles (mg/m^3)	0.252 ± 0.005^{a}	0.230 ± 0.005^{b}

Differing superscripts within a row indicate a significant difference (P<0.05)

In section A (restocked within 24 hours), 53.6% of the pens were wet (water/urine), compared to 23.2% for section B (restocked after 3 days). In section A, 46.4% of the pens had manure on the pen floor, compared to 20.4% for section B (Table 5.4).

The trends observed at 6 weeks post-stocking were observed 14 days later at 8 weeks post-stocking. All air quality parameters were reduced (P<0.01) in Section B (restocked after 3 days) compared to Section A (restocked within 24 h). All air quality parameters were reduced to below maximum acceptable levels except viable airborne bacteria.

The mean average daily gain (ADG) for the pigs raised in section A (restocked within 24 h) was 612 g compared to 643g for section B (restocked after 3 days).

It was noted that the stocking density was 2.8 m^3 airspace/pig, 0.2 m^3 airspace/pig less than the recommended level (Cargill and Banhazi, 2002).

Significant differences in respirable particles, bacteria and ammonia were observed between Section A and Section B at both time points, 6 weeks post-stocking and 8 weeks post-stocking, however, no significant differences in air quality parameters were detected between the six and eight week sampling times.

5.5.8 *Discussion*

The results suggest that there is a strong influence between pen hygiene, ammonia levels, bacteria levels, and respirable dust on growth rate.

Although the reasons for poor dunging patterns are incompletely understood, overcrowding, draughts or air movement over the pens, and wet floors are known to be

significant causes (Banhazi *et al*., 2000). To achieve and maintain good dunging patterns, it is essential that floors are dry before pens are re-stocked and that all draughts are eliminated.

Using disinfectants following cleaning has been shown to have a positive effect on subsequent hygiene (Madec and Leon, 1999; Arboleda *et al.,* 2001), especially on old and cracked floors. Many disinfectants are inactivated by organic material, such as dung, hence cleaning must be thorough (Cargill and Banhazi, 2002). In a study by Crowe *et al*., (1994) it was found that nursery pigs reared in an all-in/all-out (AIAO) management system were heavier at the end of the growing phase compared to littermates raised in a traditional farrowing system. The AIAO environment had less dust and endotoxin levels. The low levels of pollutants in the AIAO environment were achieved by rigorous cleaning and disinfection of the facilities between batches. A possible explanation for the improved growth is a decreased stimulation of the immune system, allowing more energy to be diverted to muscle growth. A study by Currie *et al.,* (1997) demonstrated significantly lower ammonia and carbon dioxide concentrations and lower total dust content in a clean environment compared to a dirty environment which resulted in a 10% improvement in daily gain. The study demonstrated that frequent cleaning of weaner pig accommodation and effluent flushing with fresh water improved air quality and pig performance. There was a trend for bacteria to be reduced from 197,000 cfu/m^3 to 138,000 cfu/m^3, in a dirty versus clean environment, however this was still above the recommended maximum level (100,000 cfu/m^3).

The improvements in growth rate and air quality parameters observed when pens are cleaned thoroughly are lost if the pens are still wet when the next batch of pigs is introduced.

The mean improvement in growth rate during the 8 week period between Section B and A was 31 g/pig/day or approximately 1.7 kg/pig, totalling 510 kg for 300 pigs. Depending on market value (eg. $2/kg) this could result in approximately $1,000, or more to the producer, just by ensuring the pens are left to dry completely before restocking. This amount could increase further if stocking density is maintained at 3.0 m^3 airspace/pig or more. Another way to look at this is the mean improvement in growth rate during the 8 week period between Section B (643 g) and Section A (612 g) was 31 g. The pigs from Section B would take 155 days to get to market, whereas the pigs from Section A would take 163 days, a difference of 8 days. If we subtract the 3 days waiting for the floors to completely dry after cleaning between batches, this leaves 5 days. This could mean that the farmer has 5 days where he/she is not spending money on feed, electricity, water and labour. This period also allows time for maintenance of the shed.

As both rooms were identical, it can be assumed that a dry pen prior to re-stocking will lead to better dunging patterns, less manure on the solid floor in the pen, and less of the solid floor area being wet with urine. This resulted in improved air quality, notably a reduction in bacteria and ammonia gas.

As the design and dimensions of section A and B were comparable, and stocking rate and stocking density were the same, it is reasonable to conclude that the shed effect on the results was due to the animals. Hence, the major contributing factors to the differences observed between sections was the pen environment.

This experiment demonstrated that by simply waiting for the pens to be dry before stocking, improvements could be made in floor wetness, floor dunging, air quality and ultimately, average daily gain.

5.6 Case study three – the effect of slat type and pit depth on air quality parameters

5.6.1 *The farm*

The farm in this study (Farm 18) had two grower-finisher, naturally ventilated sheds with similar stocking density and stocking rate. One of the sheds had a partially slatted (Shed A) and the other shed had a totally slatted (Shed B) floor. The distance between the slats and the bottom of the pit was 400 mm in Shed A and 250 mm in Shed B.

The aim of this study was to evaluate the effect of pit depth on air quality parameters following flushing.

5.6.2 *Materials and methods*

Concentrations of bacteria and ammonia were measured before and during flushing and 1, 2 and 4 hours post-flushing. To reduce the effect of total versus partial slat, all measurements were taken directly above the middle of the slats (Shed A) and in the middle of the pen (Shed B). Measurements were taken at both slat level and 0.5 metres above the slats and the sheds were flushed with recycled water. Each assessment was repeated daily over a three-day period at 12 noon each day. The side curtains were opened half way during the sampling period to avoid variation in the ventilation rate.

5.6.2.1 Ammonia and carbon dioxide

Short-term measures of ammonia and carbon dioxide gas concentrations were measured before, during and 1, 2 and 4 h post-flushing at slat level and pig breathing level (0.5 m above slat level), as described in 5.4.2.1.

5.6.2.2 Bacteria

Total viable airborne bacteria were measured measured before, during and 1, 2, 4 h post-flushing at slat level and pig breathing level (0.5 m above slat level), as described in 5.4.2.3.

5.6.3 *Results*

Table 5.5: Mean ammonia concentrations (ppm) at two sites (slat level and 0.5m above slat level) during flushing of sheds with different proportions of slats and pit depths. Data are mean values ± SEM.

Time	Shed A		Shed B	
	0 m	0.5 m	0 m	0.5 m
– 60 min	3.33 ± 0.33	2.33 ± 0.33	5.33 ± 0.88	4.33 ± 0.33
flushing	8.00 ± 0.58	5.00 ± 0.58	15.33 ± 0.88	11.67 ± 0.67
60 min	4.00 ± 0.58	2.67 ± 0.33	11.00 ± 1.73	9.33 ± 0.88
120 min	3.00 ± 0.58	2.00 ± 0.01	7.33 ± 0.88	5.67 ± 0.67
240 min	2.33 ± 0.33	2.00 ± 0.01	6.33 ± 0.88	4.33 ± 0.66

An hour prior to flushing, ammonia levels were below the maximum acceptable limit of 10 ppm, but were higher in the fully slatted shed (Shed B) (Table 5.5). The highest levels of ammonia were recorded during flushing which has also been demonstrated by Banhazi and Cargill (1999); however levels did not get above 10 ppm in the partially slatted shed (Shed A). Ammonia levels dropped to acceptable levels 2 h post flushing in Shed B.

Table 5.6: Mean bacteria concentrations (cfu/m^3) at two sites (slat level and 0.5m above slat level) during flushing of sheds with different proportions of slats and pit depths. Data are mean values ± SEM.

Time	Shed A		Shed B	
	0 m	0.5 m	0 m	0.5 m
– 60 min	127500 ± 5164	112600 ± 1021	121467 ± 2136	118467 ± 1991
flushing	152467 ± 3012	125500 ± 1184	143267 ± 1937	148667 ± 2917
60 min	123000 ± 2165	116800 ± 2386	141500 ± 2311	152733 ± 2652
120 min	121867 ± 2011	107800 ± 2020	135833 ± 3091	140033 ± 2887
240 min	117533 ± 1978	101967 ± 1567	126400 ± 2281	123767 ± 2206

All bacteria levels recorded, except one, were above the maximum acceptable level of 100,000 cfu/m^3. The highest level of bacteria for all sheds were recorded during flushing, except for the recordings for bacteria at 0.5 m in section B where the highest levels of bacteria were recorded 60 min post flushing (Table 5.6).

In the fully slatted shed (section B), the bacteria levels were higher when recorded at 0.5 m compared to slat level during flushing, and 60 min and 120 min post-flushing. This trend was reversed at 240 min post-flushing. This was not observed in the partially slatted shed (section A). In the partially slatted shed (section A) all of the bacterial levels were higher at slat level compared to 0.5 m above the slats.

5.6.4 *Discussion*

The differences observed for bacteria and ammonia concentrations between sheds would suggest that sheds with deeper pits and partially slatted floors perform best in terms of air quality, both following, and during, flushing.

While the study would have been strengthened if 2 sheds with either partial or total slatted floors were compared, rather than a mix, this was not possible. Pig sheds in Australia tend to have either total slats and shallow pits, or partial slats and deeper pits. However, the fact that measurements were taken directly above the middle of a row of slats (for partially slatted pens) or in the middle of the room (for fully slatted pens), eliminated the effect of slat area, especially at slat level. However, this may explain why bacterial levels were higher at 0.5 m than slat level in totally slatted sheds, as there may have been a contribution from the other row of slats, whereas a partially slatted

floor, the contribution was from the one row of slats. The data, however, do support previous research (Madec and Leon, 1999), that pit depth needs to be greater than 250 mm, and possibly as deep as 400 mm.

5.7 Case study four – the effect of fresh vs recycled water during flushing on ammonia and bacteria levels

5.7.1 *The farm*

The sheds assessed on this farm (Farm 19) were two grower-finisher, naturally ventilated sheds with similar stocking density and stocking rate. Both sheds had partially slatted floors and the distance between the slats and the bottom of the pit was 450 mm. The side curtains were opened half way during the sampling period to avoid variation in the ventilation rate.

Recycled water was obtained via a 3-stage gravitational sedimentation settling pond system, whereby water from the piggery sheds flowed via channels into the settling ponds. Solid particles from the water would settle in the bottom of the ponds, while water container fewer particles would over-flow into ponds, two and three via gravity. Surface water from the third pond was pumped into a holding tank next to the piggery shed. The water was not analysed for ammonia or bacteria levels.

The aim of this study was to assess the effect on air quality parameters when fresh *vs* recycled water was used to flush pits.

5.7.2 *Materials and methods*

The sheds were visited on two occasions. On the first visit, both sheds were flushed with recycled water, as per normal management practices for this farm. On the second visit, one shed (Shed A) was flushed with clean water, and the other shed (Shed B) was flushed as per normal with recycled water. Concentrations of bacteria and ammonia were measured one hour prior to flushing, during flushing, and 1, 2 and 4 h post-flushing. Measurements were taken 0.5 m above the slats, and each measurement was repeated daily over a three day period at 12 noon.

5.7.2.1 Ammonia and carbon dioxide

Short-term measures of ammonia and carbon dioxide gas concentrations were measured before, during and 1, 2 and 4 h post-flushing at pig breathing level (0.5 m above slate level), as described in 5.4.2.1.

5.7.4 Bacteria

Total viable airborne bacteria were measured measured before, during and 1, 2, 4 h post-flushing, as described in 5.4.2.3.

5.7.5 *Results*

Table 5.7: Ammonia and bacteria concentrations 0.5 m above the slats during flushing of sheds with recycled water. Data are mean values ± SEM.

Time	Ammonia (ppm)		Bacteria (cfu/m^3)	
	Shed A	**Shed B**	**Shed A**	**Shed B**
- 60 min	5.00 ± 0.58	5.33 ± 0.33	111500 ± 1078	112333 ± 1684
flushing	13.00 ± 2.31	11.67 ± 2.33	147800 ± 1053	147500 ± 2572
60 min	9.00 ± 1.53	9.33 ± 1.85	139667 ± 4821	135667 ± 4848
120 min	7.00 ± 1.53	8.33 ± 1.85	124533 ± 606	125333 ± 3426
240 min	4.67 ± 0.88	5.00 ± 1.00	110200 ± 1081	110000 ± 2219

Table 5.8: Ammonia and bacteria concentrations 0.5 m above the slats during flushing of sheds with fresh (Shed A) and recycled water (Shed B).

Time	Ammonia (ppm)		Bacteria (cfu/m^3)	
	Shed A (fresh)	**Shed B (recycled)**	**Shed A (fresh)**	**Shed B (recycled)**
- 60 min	3.00 ± 0.58	4.67 ± 0.33	107803 ± 569	110643 ± 416
flushing	8.67 ± 1.45	12.67 ± 1.45	123700 ± 2272	150100 ± 1779
60 min	5.33 ± 1.20	10.33 ± 1.33	115767 ± 1386	141733 ± 1924
120 min	3.33 ± 0.88	8.33 ± 1.33	107233 ± 548	130800 ± 1114
240 min	2.00 ± 0.01	5.33 ± 1.33	100700 ± 1365	110933 ± 788

All bacteria levels were above the recommended acceptable level of 100,000 cfu/m^3. As expected from Case Study 3 data, bacteria levels were highest during flushing with recycled water and decreased over the next 4 hours post-flushing.

During the first visit, ammonia levels were similar 0.5 m above the slats (Table 5.7). The highest levels of ammonia were recorded during flushing which was expected from Case Study 3.

When the pits were flushed with fresh water (Shed A), bacterial levels were highest during flushing but were lower than when the pits were flushed with recycled water (Table 5.8). Bacteria levels did reduce to acceptable levels 4 h post-flushing when fresh water was used (Shed A).

During the second visit when Shed A was flushed with fresh water, ammonia levels did not reach 10 ppm. However, ammonia levels in Shed B averaged 10 ppm or greater.

5.7.4 *Discussion*

This experiment demonstrated that the use of fresh or recycled water had its greatest effect on ammonia levels. There was an improvement in bacteria levels, but they generally were above the maximum acceptable limit of 100,000 cfu/m^3.

Similar results were observed in a study by Currie *et al*., (1997) in which a dirty environment was created by passing recycled water beneath the floor slats and not

cleaning the room throughout the experiment. Ammonia and bacteria levels increased (P<0.01) when recycled water was used versus fresh water, which was associated with a 10% decrease in average daily gain.

Elevated concentrations of ammonia gas and bioaerosols in the airspace of naturally-ventilated pig sheds have been shown to have a negative effect on the health and growth rate of growing pigs (Donham, 1991; Cargill and Skirrow, 1997).

Flushing pits with fresh water had a positive effect to decrease levels of ammonia and bacteria in the airspace of the sheds. Some piggeries in Australia use recycled water because fresh water is limited or because the use of fresh water adds to costs substantially. The results from this Case study suggest that sheds should be ventilated well during the flushing period, providing thermal comfort is not compromised.

Although it would have been good to determine the growth rate data for the pigs housed in these sheds, this was not feasible as the clean water flushing trial was only used for 3 days during the experiment.

6

General discussion and conclusions

6.1 General discussion and conclusion

The observations reported in this thesis provide a better understanding of the importance of air quality and its effect on the growth rate of pigs. A number of strategies to reduce airborne pollutants and improve air quality in pig sheds have been identified.

The aims of this study were to:

- investigate the effects of ammonia and bacteria on feed intake, immune function and physiology of the respiratory tract of pigs;
- investigate the effects of stocking density on selected air quality parameters (ammonia, dust and bacteria) and growth rate in pigs;
- investigate the effects of improving shed design and management on air quality parameters (ammonia, dust and bacteria) and growth rate in pigs;
- test and validate a number of strategies for reducing selected air pollutants in pig buildings, including slat type, pit depth, time taken to re-stock pens and the use of fresh *vs* recycled water.

The study reported in Chapter 3 was designed to determine the effects of ammonia gas and alpha haemolytic cocci (AHC) on feed intake, immune function and physiology of the respiratory tract of healthy female pigs. This study was novel in that individual animals (16 week-old gilts) were challenged with ammonia, AHC or a combination of both, for a period of 14 days. The concentration of ammonia and bacteria used were consistent with those that have been observed in Australian piggeries.

This study demonstrated that significant effects on production and immune function could be achieved during the 14 day trial period. Although AHC appear to have a greater effect than ammonia on growth rate and feed efficiency, as well as aspects of immune function, the largest effects were observed in pigs exposed to high levels of ammonia followed by AHC, so that growth rates were reduced by 1.8, 3.1 and 4.9% when pigs were exposed to 10, 25 and 50 ppm ammonia, respectively, whereas growth rates were reduced by 12.8, 26.5, 35.2 and 47.2% compared to controls when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/m^3), respectively.

Feed conversion efficiency was reduced by 1.3, 0.4 and 2.1% compared to controls when pigs were exposed to 10, 25 and 50 ppm ammonia, respectively. Feed conversion efficiency was reduced by 7.4, 16.0, 20.0 and 30.2% compared to controls when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml), respectively.

Feed intake was reduced by 0.8, 4.2 and 2.7% compared to controls when pigs were exposed to 10, 25 and 50 ppm ammonia, respectively. Feed intake was reduced by 6.9, 13.0, 20.6 and 25.6% compared to controls when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml), respectively.

The lymphocyte stimulation increased by 16, 16, 17 and 25% during the 14 day trial period when pigs were exposed to 0, 10, 25 and 50 ppm ammonia, respectively. Lymphocyte proliferation increased by 31, 49, 67 and 76% compared to controls when

pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml), respectively.

The phagocytosis activity increased 17, 17, 20 and 20% during the 14 day trial period when pigs were exposed to 0, 10, 25 and 50 ppm ammonia, respectively. The phagocytosis activity increased by 46, 60, 71 and 78% compared to controls when pigs were exposed to 0, 10, 25 and 50 ppm ammonia plus AHC (200,000 cfu/ml), respectively.

The observed reductions in growth rate and feed conversion efficiency, as well as the increase in lymphocyte proliferation and phagocytic activity, suggest that there had been a non-specific activation of the immune system. Immune system activation involves the production of cytokines and is thought to divert nutrients away from growth and accretion of skeletal muscle to support the inflammatory and immune responses (Almond *et al*., 1996; Johnson, 1998; Le Floc'h *et al*., 2006).

The findings from this study are consistent with studies in pigs and other species, which indicate that airborne particles engage the immune system, initiating physiological changes resulting in reduced growth and performance. Previous studies in nursery and weaner pigs have suggested that pro-inflammatory cytokines correlate with low feed intake and growth (Spurlock, 1997; von Borell *et al*., 2007). Drummond *et al*., (1980) exposed 4-week old pigs to 50, 100 and 150 ppm ammonia and reported a reduction in ADG of 12%, 30% and 29%, respectively, compared to controls. The 16-week old pigs

in my experiments recorded a non-significant (P<0.05) reduction in ADG compared to control animals of 1.8%, 3.1% and 4.9% when exposed to 10, 25 and 50 ppm ammonia. This is consistent with the findings of Wathes *et al*., (2004), who exposed weaned pigs for 5.5 weeks to ammonia at concentrations of 0, 10, 20 and 40 ppm and were unable to show a significant difference in ADG between the groups. Von Borell *et al*., (2007) exposed weaned pigs for 20 days to ammonia at concentrations of 0, 35 and 50 ppm. Although ammonia was shown to elicit increases in WBC, absolute numbers of lymphocyte and monocytes, and serum cortisol and haptogloblin, they were not able to detect effects on growth performance, other than a trend toward low dry matter intake at 50 ppm ammonia exposure.

In this sudy, exposure to 10, 25 and 50 ppm ammonia was tolerated by the pigs with no trend for differences observed in their feed intake. There were no signs of aversion to ammonia gas at any concentration (10, 25 and 50 ppm), with the majority of feed being consumed within 30 minutes. A study by Wathes *et al*., (2002a) reported a delayed aversion to high concentrations (20 and 40 ppm of ammonia) in weaner pigs, which they attributed to a gradual development of a sense of malaise. A study by Jones *et al*., (1998) exposed pigs to ammonia at either 40 or 100 ppm and found that pigs prefer to eat rather than avoid exposure to ammonia.

When pigs were exposed to 0, 10, 25 or 50 ppm ammonia plus AHC (200,000 cfu/m^3) feed intake decreased compared to control animals. Wathes *et al*., (2004) exposed pigs to ammonia at 0, 10, 20 and 40 ppm and an ‘artificial’ dust (mixture of food, barley

straw and faeces) and found a small, but significant, reduction in live weight gain relative to controls when dust levels increased to 5.1 and 9.9 mg/m^3, however, there were no differences in food efficiency. Contrary to this, the results from my study did show a significant reduction in feed efficiency when pigs were exposed to ammonia and bacteria. While a reduction in feed intake can account for some of the decrease in weight gain compared to controls, the increase in FCR would indicate that energy is being diverted from muscle growth, and re-directed towards tissues involved with the inflammation and immune response (Almond *et al*., 1996; Johnson, 1998; Sandberg *et al*., 2007).

One explanation of the reduced growth rates observed in my trial could be the production of specific factors, called cytokines. Cytokines are produced and secreted by the pig's white blood cells as a defence mechanism in response to the presence of endotoxins. Cytokines suppress the secretion of the significant growth promoting hormones, affect blood glucose homeostasis, increase protein oxidation, increase muscle proteolysis and alter other metabolic processes (Almond *et al*., 1996; Johnson, 1998). Thus, immunological challenge impairs metabolism intended for growth and skeletal muscle accretion in order to enhance metabolic processes that support the immune response. The alteration in metabolism involves a decrease in IGF-1 concentrations. It is for this reason that dietary manipulation fails to improve pig growth after immunological challenge (Black *et al.,* 2001).

The influence of immune stimulation on growth has been well documented in the poultry industry (Kelley *et al*., 1987; Klasing and Barnes, 1988). In chickens, immune stimulation reduced weight gain, increased muscle protein degradation, decreased protein synthesis, and reduced muscle protein accretion. Birds reared in environments free of airborne pollutants grew 25% faster than birds in commercial conditions (Butler and Egan, 1974). In another series of studies it was found that when chicks were exposed to *E. coli* aerosols alone, no effects on respiratory tissues or the health of the birds could be observed. However, when high levels of sterile dust (100 mg/m^3) or ammonia were included with the *E. coli* aerosols, inflammatory changes in respiratory tissues were evident (Oyetunde *et al*., 1978). Studies by Kent *et al*., (1992) in humans demonstrated that recombinant IL-1, injected either peripherally (i.p) or centrally (i.c.v), increased oxygen consumption and body temperature, reduced motivation for food and decreased interest in social activities. These results confirmed that IL-1 is a potent initiator of fever and anorexia.

The study reported in Chapter 4 was designed to investigate the effects of stocking density (m^3 airspace/pig) on air quality parameters and the growth rate of pigs. This study was novel in that data were obtained from individual batches of pigs from different farms in different states of Australia. While there may have been some genetic variation and nutritional differences in pigs on farms 2 to 9, the pigs from the single stage grower units (Farms 10 – 15) were of the same genetic stock, fed the same diets, and housed in similar sheds, hence, these were not confounding factors. The only difference was the stocking density. On the other farms (2 – 9), multiple (8) batches of

pigs were monitored to reduce the confounding effects of genetics, nutrition and management.

The findings from Chapter 4 indicate a strong positive relationship between the stocking density (m^3 airspace/pig) and the mean growth rate of pigs from 10 to 22 weeks of age, in an all-in/all-out (AIAO) system. There was also a strong negative correlation between stocking density (m^3 airspace/pig) and the number of viable bacteria in the airspace, so as the concentration of bacteria in the airspace increased, the growth rate of the pigs significantly declined. In fact, the data from this trial suggest that high concentrations of airborne bacteria may be one of the major drivers for reduced growth rate in association with a high stocking density (m^3 airspace/pig).

These observations are consistent with previous studies in Australia, leading to recommendations for naturally ventilated sheds of 2.6 m^3 airspace/pig for pigs weighing 60 kg and 3.0 m^3 airspace/pig for pigs weighing 100kg pigs (Pointon *et al*., 1995). In other studies it has been shown that overcrowding (high stocking density) is also associated with poor dunging patterns, which in turn reduce hygiene and air quality standards (Banhazi *et al.,* 2000) and pen hygiene has been identified as a risk factor for enteric disease (Madec and Leon, 1999).

In an earlier study into the causes and risk factors associated with pleurisy in pigs on Australian farms, it was found that although a range of pathogens were involved, the prevalence of pleurisy in a herd was associated directly with a number of husbandry and

environmental factors (Skirrow *et al*., 1995). The most significant factors associated with increased pleurisy prevalence were the concentration of airborne streptococcal organisms present in the shed and the concentration of airborne respirable dust. Other significant factors included the stocking density (m^3 airspace/pig), and the number of pigs sharing the same airspace (Skirrow *et al*., 1995). It was also found that stocking density levels were above the recommended level in a majority of farms. The authors also reported that the number of pigs in an airspace (shed population) was not only positively correlated with the concentration of airborne respirable dust, but also with the bacterial load in the airspace, and the prevalence of pleurisy, pneumonia and coughing rates in pigs (Skirrow *et al*., 1995). On the other hand, the volume of airspace per pig was negatively correlated with the bacterial load in the airspace, the concentration of airborne streptococcal organisms and pleurisy prevalence.

The case studies in Chapter 5 were undertaken to determine the effects of shed design and management on air quality parameters and growth rate. Although these were only case studies on individual farms, the results indicate that improving ventilation through widening ridge vents, leaving floors to dry before re-stocking pens, increasing pit depth to around 400mm, and flushing pits with fresh water will all have a positive effect on air quality parameters and growth rate.

The case studies also indicate that management of air quality and shed hygiene in existing sheds can represent a challenge, as major renovations may be needed to achieve improvements. In the first case study, substantial improvements in both production

(g/day) and air quality parameters were achieved by widening the ridge vent and raising the ridge vent cap to improve ventilation. Other studies in Australia have targeted the key building, husbandry and environmental factors that increase levels of pollutants, with emphasis on factors that have a negative influence on surface and air hygiene (Cargill and Banhazi, 2002).

Case study 1 demonstrated that widening the ridge vent and increasing the stocking density improved ventilation and air quality, which was associated with improved growth rates. The results observed in this trial are similar to those in a study by Cargill *et al*., (1999) in which the authors renovated existing sheds, which included increasing ridge vents, adding side walls, and adhering to the recommended stocking rates and stocking density. Air quality parameters (ammonia, dust and bacteria) decreased and this was associated with an improvement in average daily gain from 560 g to 628 g, an increase in dressed weight from 67 kg to 70 kg and a reduction in days to market from 184 to 172. The cost for these renovations ranged from $1,600 to $2,950, while the cost:benefit ratio ranged from 0.27 to 1.28. A value of 0.27 indicates that the renovations would have 'paid for themselves' after 4 batches of pigs. It is important to remember that the renovation costs are 'one off' and the benefits in air quality and growth rates will be sustained for as long as best practice management procedures are followed.

The importance of dry floors, dunging patterns and hygiene was evident in case study number 2 when pens were re-stocked while still wet, or allowed to dry. This study

indicates the importance of keeping floors as dry, and as free of dung as possible to reduce air pollutant levels and maximise growth rate.

The production benefits gained by improving floor hygiene in Case Study 2 have also been demonstrated in a study by Cargill and Banhazi (1998) where pigs reared in cleaned rooms grew from 8 to 10% faster than pigs reared in uncleaned or dirty rooms. Other studies also reported that maintaining a high standard of hygiene resulted in increased growth rates (Knowles *et al*., 1997; Lee *et al*., 1997; Le Floc'h *et al*., 2009). As expected, when pigs were housed in a clean environment where all air quality parameters were at least 10% below target maximum levels, pigs housed in single pens grew 38 g/day faster than group penned pigs (10/pen). However, in the dirty environment, where concentrations of dust, bacteria and ammonia were 50 to 100% above target levels, there was no difference in growth rate between single penned and group penned pigs. Single penned pigs in the clean environment grew significantly faster (77 g/day) than the pigs in the dirty environment, as did group penned pigs in the clean environment. Hence, a dirty environment not only reduced growth rates, but also eliminated the positive effect of housing pigs in single pens. Significant differences were also recorded in ammonia, dust and carbon dioxide levels between the sections (Currie *et al*., 1997) and neutrophil function, lymphocyte proliferation and plasma concentrations of acute phase proteins were significantly higher in pigs reared in the dirty environment (Black *et al*., 2001).

Effluent is a major source of key airborne pollutants, hence factors such as the type of

effluent system, the use of recycled water, and the distance between the surface of the slurry and the base of the slats (Madec and Leon, 1999) all influence air and surface hygiene. Broken and blocked slats, as well as air entering the shed through openings over the pits at the end of the shed, will exacerbate the problem.

In both case studies 3 and 4 the negative effect of flushing pits on air quality was clearly demonstrated. However, the data also suggest that increasing pit depth to around 400 mm will reduce both the level of pollutants during and immediately following flushing, as well as over time and maintaining a higher standard of air quality. The data from case study number 4 also demonstrate the negative effect of using recycled water compared with fresh clean water for flushing pits. However, the study failed to provide economic values supporting evidence to justify the use of clean water.

The results from the case studies confirm that there are several housing and management factors that influence production efficiency through air and surface hygiene. These include shed volume, which affects stocking density, the size of the ridge vent and sidewall shutters (in naturally ventilated sheds), which influences ventilation rates, as well as the depth of effluent channels which influences air pollution. Assessment of air quality in a large number of sheds in Australia indicates that the maximum width of a naturally ventilated shed should not exceed 12 metres. The evidence suggests that 10 metre wide sheds perform the most efficiently. In 26 sheds assessed for air quality, the correlation between shed width and the concentration of bacteria was -0.54 ($P<0.01$) (Cargill and Banhazi, 2002). Shed height determines the

amount of airspace provided, as well as the angle of the roof pitch and the space available for sidewall openings. All of these factors influence ventilation rate (Cargill and Skirrow, 1997) in naturally ventilated buildings. Other factors include the width of the ridge vent and the height of the ridge cap. It is recommended that sidewall openings should be a minimum of 20% of the width of the shed, and the width of the ridge vent at least 10% of the width of the shed. The recommended angle for the roof pitch is a minimum of 15 degrees (Cargill *et al.,* 1999).

Adopting more innovative management systems is essential for improving air and surface hygiene in both new and existing sheds (Crowe *et al*., 1994; Cargill *et al*., 1995; Cargill *et al*., 1996; Cargill *et al*., 1998, Cargill *et al*., 2000; Banhazi and Cargill, 1998; Banhazi *et al.*, 1999; Madec *et al*., 1998; Madec and Leon, 1999). Systems that incorporate all-in/all-out (AIAO) management, and cleaning facilities between batches, must be regarded as 'best practice' in terms of maximising hygiene and air quality. My observations are consistent with a number of reports demonstrating that systems using AIAO production methods result in significant improvements in air quality in existing sheds (Cargill and Banhazi, 1996; Cargill *et al*., 1998; Banhazi *et al*., 1999; Cargill *et al*., 2000) and a high standard of hygiene can also be maintained, provided good dunging patterns are achieved (Banhazi *et al*., 2000). Slats, leaking pipes and drinkers, and damaged feeders can all be repaired when sheds are empty.

6.2 Summary of conclusions

While a range of pollutants such as gases and particulate matter and toxins are present in the airspace of animal houses, the important ones identified in this study in terms of reduced growth rates appear to be ammonia and bacteria. While clinical signs may include coughing, sneezing, salivation, loss of appetite and excessive lachrymal secretions, the key finding in this study was reduced growth rates in association with immune stimulation. It is important to note that these changes were not limited to pigs with respiratory disease. However, other studies have also shown that as well as activation of the immune system, both local and generalised inflammatory responses occur (Black *et al*., 2001), as well as activation of the immune system. Local inflammatory changes include loss of cilia, thickened epithelia and decreased numbers of goblet cells in the trachea and turbinates. It also involves activation of epithelial cells, alveolar macrophages, and polymorphonuclear cells, which then release a variety of inflammatory mediators. The non-specific activation of the immune system involves the production of cytokines and is thought to divert nutrients away from growth and accretion of skeletal muscle to support these immune and inflammatory responses. As a result, reduced air quality is one of the major factors preventing intensively housed pigs from reaching their maximum growth rate potential and reducing immune competence.

This study clearly identifies cleanliness and building hygiene as important factors affecting air quality and pig health, and provides a number of strategies to rectify problems. It is clear that facilities need to be managed as an all-in/all-out (AIAO) system, as this enables farmers to maximise hygiene by thoroughly cleaning pens

between batches. A good cleaning regime includes removing all dung, applying a degreasing agent, using a high pressure water hose (ideally using hot water) to clean all surfaces, and fogging the room with a powerful disinfectant (Banhazi *et al*., 2003). As seen in Case Study 2, it is important that pens are completely dry before the next batch of pigs move in. It is also important that when pigs are moved into the clean pens, and throughout their growing phase, that stocking rates and stocking density are kept to the recommended levels. Overcrowding can 'undo' all the benefits achieved with correct dunging patterns and cleaning.

This study was unique, in that it was able to demonstrate a successful model to expose individually-housed pigs to individual, or combinations, of airborne pollutants without the need for 'exposure chambers'. In particular, the study was able to determine the effects of ammonia and alpha haemolytic cocci (AHC) on individually-housed pigs. The results demonstrated that AHC in the absence of ammonia elicited an immune response and depressed growth and feed utilisation parameters, and hence AHC are not commensal. The impacts of AHC are markedly exacerbated by exposure to ammonia, but even though the condition remains sub-clinical, there are impacts on growth and feed utilisation. This study demonstrated that viable AHC contribute to the impact of poor pig shed hygiene on production parameters.

As mentioned in the introduction, one of the aims of a piggery is to have a cost-efficient production without compromising the welfare requirements of the pigs and those working with pigs. The implication is that if sheds and pigs are managed with a focus

on hygiene, the air quality will improve, the pigs' immune system will not be activated, disease prevalence will decrease, feed conversion will improve, and average daily gain will be maximised. While this is important for the pigs within the shed, it is also of relevance for the health of stockpersons and others working with the pigs. Improving and maintaining a good level of air and surface hygiene will help the piggery enterprise become more profitable.

7

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